

SPACELAB LIFE SCIENCES 1

REPRINTS OF BACKGROUND LIFE SCIENCES PUBLICATIONS

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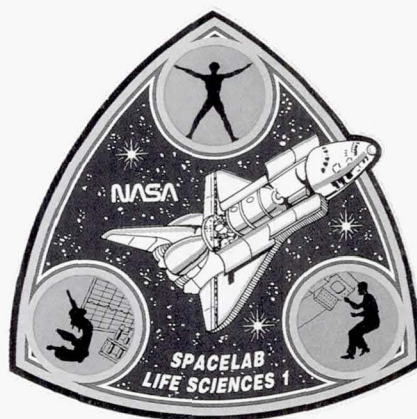
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Preface

The first Spacelab mission dedicated to life sciences research (SLS-1) is scheduled to be launched in 1991. Almost all the investigations to be conducted on this mission were originally proposed in 1978 and selected by NASA in 1981. Thus, the planning of this mission spans more than a decade. During this time, NASA has funded the investigator teams not only to prepare the experiment for flight, but to conduct the ground-based research to support this effort. This book is an attempt to summarize this background and supportive research by publishing selected papers and abstracts of the SLS-1 investigators.

The research being conducted on SLS-1 is primarily concerned with a common theme, the short-term adaptation of physiological systems to weightlessness. Both human and animal studies are being conducted. In most cases, the animal studies are designed to validate the use of animals as models for studying human adaptation. The physiological systems to be studied include: cardiovascular and cardiopulmonary, musculoskeletal, neurovestibular, renal and endocrine, hematological, and immunological.

This volume is composed of two sections. The first section consists of selected research from the 18 SLS-1 investigator teams, presented in their entirety. These papers provide a comprehensive overview of the various disciplines being studied on SLS-1, in particular with respect to gravitational effects and adaptation to space flight. The second section contains citations and abstracts of all the papers submitted by the SLS-1 investigator teams. It is intended to be a reference source for additional, often more detailed, information than provided by the papers in the first section.

This compendium of papers and abstracts is intended to serve two audiences, the active researchers interested in space biology and medicine, and the graduate student who may be taking one of the excellent survey courses currently available in the space life sciences. The editors hope that the readers will find this book of pre-SLS-1 research to be useful and of interest. We expect to publish a companion volume when the results of the SLS-1 mission have been reported.

SECTION I - REPRINTS

This section presents selected research papers from the various disciplines being studied on SLS-1. The large volume and broad scope of the research represented by the SLS-1 investigator teams made selection of these papers challenging. The following guidelines were used to choose this small group of papers: a) at least one paper would be included from each of the 18 SLS-1 investigator teams; b) at least one paper would be included from each of the major life sciences disciplines that are represented in the SLS-1 payload; c) the papers should provide an overview of the particular research discipline being addressed; and d) if possible, the selected papers should be concerned with gravitational effects or adaptation to space flight.

The selected papers provide a comprehensive overview of each investigator team's field of research, in particular with regard to the SLS-1 mission experiments. It is hoped that presentation of these papers will also provide readers with an appreciation of the scope of the investigations performed on SLS-1.

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THE ROLE OF CALCIUM IN
OSTEOPOROSIS

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KEY WORDS: bone, nutrition, calcium intake, calcium supplementation, physical activity

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INTRODUCTION

Osteoporosis is a complex disorder of multifactorial origin that is characterized by an asymptomatic reduction in the quantity of bone mass per unit volume (4). When bone mass becomes too low, structural integrity and mechanical support are not maintained, and fractures occur with minimal trauma. The most common sites of osteoporotic fracture are the proximal femur, distal radius, vertebra, humerus, pelvis, and ribs. In clinical research, such a diagnosis is frequently applied only to patients with one or more fractures (76) even though it may be detected in many patients by measuring

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bone mass with single or dual photon absorptiometry (SPA or DPA) (71) or with quantitative computerized tomography (QCT) (32).

Osteoporosis occurs most frequently in postmenopausal white women and in the elderly (14). About 20% of women suffer osteoporotic fractures by age 65, and more than 30% sustain fractures by age 90 (65). Significant osteoporosis is not observed in men and black women until after age 60, when fracture rates progressively increase.

BONE AND MINERAL METABOLISM

Bone is composed primarily of calcium and phosphorus in the form of hydroxyapatite crystals deposited in a collagen matrix (121). There are two types of bone in adults, cortical and trabecular (4). Cortical bone provides rigidity and is the major component of tubular bones (appendicular skeleton). Trabecular bone is spongy in appearance, provides strength and elasticity and constitutes about 60 to 70% of the vertebrae (axial skeleton).

Bone is a metabolically active tissue that turns over constantly. This process is regulated by cellular activities that resorb (osteoclastic) and form (osteoblastic) bone. In normal adult bone, resorption is coupled to and exactly balanced by formation so that no net change in the amount of bone is produced (26). To either increase or decrease net bone mass, these cellular processes must become functionally uncoupled, an event that is poorly understood at this time. By contrast, the major mineral ions of bone (calcium, phosphorus and magnesium) play a more passive role in bone mass changes. They must be present at physiologic concentrations in extracellular fluids for normal bone mineralization (formation) to occur (67). Dietary minerals help to maintain extracellular concentrations by replacing minerals lost to obligatory processes (urine, stool, and sweat) or those normally distributed to bone and soft tissues (35).

Maximum bone mass is achieved by about age 25–30 years, is maintained without much change until 35–45 years, and is lost at a constant rate of 0.3–0.5% per year in both men and women thereafter (35, 64, 90). For about 8–10 years immediately prior to and after menopause, most Caucasian women lose bone at a rate of 2–5% per year.

ASSOCIATION OF DIETARY FACTORS WITH OSTEOPOROSIS

Epidemiologic and Clinical Studies

CALCIUM

Absorption and balance Calcium balance generally reflects the degree of coupling of bone formation and resorption processes. Negative balances are

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recorded when resorption exceeds formation and positive balances when formation exceeds resorption. It is important to recognize that because 99% of body calcium is in bone, it is not possible to build bone without positive calcium balance nor to be in negative balance without losing bone.

Knowing the amount of dietary calcium needed to achieve "zero balance" is key to setting nutritional requirements for calcium. However, the metabolic balance technique used to determine calcium balance has important theoretical and practical limitations that can result in inaccuracies. Calcium balance depends upon a number of factors including the amount of calcium in the diet, the efficacy of calcium absorption by the intestine, and excretion of calcium. Intestinal absorption of calcium decreases with age (28, 39, 46), which may be due to an age-related decrease in serum levels of 1,25-dihydroxyvitamin D [1,25(OH)₂D₃] (120), the biologically active metabolite of vitamin D that is produced by the kidney and regulates intestinal calcium absorption (17, 85). Serum immunoreactive and bioactive parathyroid hormone (25) increase with age, which is probably a response to the age-associated decrease in calcium absorption and probably represents secondary hyperparathyroidism. It is unknown whether this endocrine adaptive response to decreased calcium absorption contributes to the decreased skeletal mass and increased incidence of fractures seen in the elderly.

Relationship between calcium intake and bone mass, osteoporosis, and fracture There are two major methodologic problems in evaluating the evidence relating calcium intake to bone mass. One is the inaccuracies inherent in determining dietary calcium by historical recall and the relation of that data to lifetime intake. Another is the inconstant use of different methods to measure bone mass; some measure predominantly cortical bone and others trabecular bone. Furthermore, methods to identify altered geometric and structural properties, so far not available, may be important to identify osteoporotic bone accurately (34, 36).

Decreased skeletal mass is the most important risk factor for bone fracture without significant trauma (35, 104). The level of bone mass achieved at skeletal maturity (peak bone mass) is a major factor modifying the risk of development of osteoporosis. The more bone mass available before age-related bone loss, the less likely it will decrease to a level at which fracture will occur (35, 90). Another major factor modifying osteoporosis risk is the rate of bone loss as life progresses.

Normally, longitudinal bone growth is complete sometime during the second decade of life. For this to occur normally, positive calcium balance is needed in the range of 110 mg/day for females and 140 mg/day for males. During the adolescent growth spurt, the required calcium retention is two to three times higher (30, 84), and the Food and Nutrition Board (76a) recommends intakes of 1200 mg/day for those 10 to 19 years of age.

Opinion is mixed as to the age at which peak bone mass is achieved. Data concerning this issue is available only from studies using cross-sectional designs (1, 30, 70, 118). They show that bone mass does not appear to reach maximum levels until sometime during the mid twenties or early thirties, or 5 to 10 years after longitudinal bone growth has ceased. It is thought that during this period cortical porosity, increased during the adolescent growth spurt, is filled in and bone cortices become thicker. The quantity of bone mass that can be added is unclear, but it is estimated to be 5–10% (90). The optimum calcium retention to achieve this apparent increment in bone mass is not yet known but is probably in the range of 40 to 60 mg/day (70). The Food and Nutrition Board incorporated this view into the latest Recommended Dietary Allowances (76a) and extended the period during which positive calcium balance must be maintained to achieve peak bone mass. Thus, the RDA for the age group 20–25 years is the same as for 10–19 years or 1200 mg/day.

Many published reports have shown either no (3, 30, 52, 56, 89, 106, 113, 114, 118) or only a modest positive relationship between bone mass and dietary calcium (especially lifetime intake) (11, 16, 31, 33, 50, 70, 92).

Nordin (83) reported the results of an international investigation of calcium intake and osteoporotic fractures. In spite of inconsistent methods in the reporting of calcium intakes by the countries involved, it was possible to demonstrate an inverse rank order relation between calcium intakes and osteoporotic vertebral fracture frequency (determined by spine X-ray). Japanese women, whose calcium intake averaged 400 mg/day, had the highest frequency of fracture, and Finland, with the highest intake (1300 mg/day), had the lowest fracture frequency. This relationship did not hold for some countries. Whereas calcium intakes in Gambia and Jamaica were low, osteoporotic fractures were rare. Recently, Holbrook et al (43) related calcium intake data collected in 1973 to subsequent hip fracture incidence among 957 Caucasian adults (50–79 years in 1973). The group suffering hip fracture had a lower nutrient density of calcium than the group without fracture. The most widely cited of the papers showing a positive effect of calcium is that of Matkovic et al (70) which reported a 5–10% greater metacarpal cortical volume in the inhabitants of a Yugoslavian district with a high calcium intake as compared with the inhabitants of a district with a low calcium intake. The inhabitants of the "high calcium" district also had a 50% lower incidence of hip fractures. In contrast, no difference was detected in the incidence of fractures about the wrist. Because the differences in bone mass as a function of age were constant, it is likely that high lifelong intakes of calcium in the high calcium district increased peak cortical bone mass rather than prevented bone loss. In contrast to these results, Riggs et al (106) found no relationship

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between the calcium intakes (range 260 to 2003 mg/day, mean 922 mg/day) of 106 normal women, age 23 to 84 years, and the rates of change in bone mineral density at the midradius (determined by single photon absorptiometry, SPA) and the lumbar spine (determined by dual photon absorptiometry, DPA) over a mean period of 4.1 years.

Most clinical studies of dietary calcium in osteoporotic patients show lower intakes than in age-matched control subjects (62, 82, 103). Whereas dietary calcium was lower than 800 mg/day in both patients and controls in all of these investigations, intakes were greater than 800 mg/day in a study in which no differences in calcium intake between osteoporotic patients and controls were demonstrated (84). The results of this latter study support Heaney's view (35) that low dietary calcium may play a permissive rather than a causative role in the development of osteoporosis and that this role can be demonstrated best when dietary calcium is below a "saturation" level.

Effects of calcium supplementation on bone mass and fracture The long-term effects of calcium supplementation on bone mass are not yet established. The results of investigations over two years or less are mixed. In general, they show a slowing of bone loss measured at sites comprised mostly of cortical bone but not at sites comprised of trabecular bone. All studies using estrogen treatment as a companion protocol have shown that calcium supplementation is inferior to estrogen in slowing cortical bone loss and that estrogen prevents trabecular bone loss completely. Some of these studies were randomized (55, 97, 98, 109, 112), but only two were blinded (109, 112). In the study of Smith et al (112) 40% of the subjects were lost to follow-up.

The results of a study performed by Recker et al (98) reflect those of the others. After two years, a supplement of 1.04 grams of elemental calcium, given as the carbonate salt, to 22 women between 55 and 65 years of age resulted in a 0.22% decrease in metacarpal cortical bone area as compared with a 1.18% decrease in 20 placebo-treated age-matched women ($p < 0.05$). By contrast, there was no difference in bone mineral content of the distal radius (mixture of trabecular and cortical bone). The effect of calcium supplementation to prevent metacarpal cortical bone loss was less than the effect of estrogen treatment in 18 age-matched women, and estrogen completely prevented bone loss at the distal radius. In a similar but nonrandomized study, Horsman et al (45) administered 800 mg of elemental calcium as the gluconate salt to 24 postmenopausal women over a two-year period and found a significant decrease in bone loss from the ulna (cortical bone) as compared to 18 placebo-treated control subjects. However, calcium treatment caused little if any diminution of the bone loss observed at the distal radius or in metacarpal cortices. Similarly, Nilas et al (79) found no change in bone

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mineral content at the distal radius when three groups of women with calcium intakes varying from below 550 mg/day to greater than 1150 mg/day were administered a 500 mg elemental calcium supplement daily. However, an investigation performed by the same group (109) which was both randomized and blinded, found that the administration of 2000 mg/day of elemental calcium as the carbonate salt for two years to postmenopausal women slowed bone loss at the proximal forearm and slowed calcium loss from the total skeleton, while the loss of bone from sites composed predominantly of trabecular bone was no different from that of placebo-treated control subjects. As in previous studies, bone mineral content remained constant at all measurement sites in subjects receiving estrogen. In a nonrandomized study, Ettinger et al (24) found no effect of calcium supplementation up to 1500 mg/day, as the carbonate salt, on bone mineral content in the spine as assessed by QCT, or on distal radius or metacarpal cortical bone mass in 44 postmenopausal women as compared with 25 age-matched women who elected not to receive treatment. By contrast, 15 women who elected to take low-dose conjugated estrogen (0.3 mg/day) combined with 1500 mg of calcium per day demonstrated complete protection against bone loss. This study and that of Cann et al (9) suggest the possibility that dietary calcium may play a permissive role in the maintenance of bone mass that is sex hormone dependent. However, Riis et al (108) found no potentiation of estrogen treatment by calcium supplementation.

Riggs et al (102) showed that the increased bone resorption surfaces observed in iliac crest bone biopsies from osteoporotic patients are restored toward normal by combined calcium and vitamin D supplementation. This effect was associated with a decrease in serum immunoreactive parathyroid hormone (iPTH) within the normal range, an event the authors justifiably speculated was responsible for the decrease in resorption surfaces. In a two-year randomized trial, severely osteoporotic women given 1200 mg/day of calcium had improved bone mineralization rates, compared to placebo-treated patients (87). The results of several other investigations, not involving bone histomorphometry, are consistent with this apparent antiresorption effect of calcium supplementation. Recker et al (98) showed that bone resorption was decreased when postmenopausal women were given supplements of calcium carbonate. Oral calcium suppresses hydroxyproline excretion, a well-established index of bone resorption, in osteoporotic postmenopausal women (44), but only under conditions of normal calcium absorption (77).

The evidence relating calcium supplementation to fracture prevalence is scanty. The only study of substance comes from the Mayo Clinic (105), reporting a nonrandomized but prospective assessment of the effect of various treatments of patients with generalized osteopenia on the occurrence of future vertebral fractures. In the study, 8 individuals received calcium carbonate

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(1500–2500 mg/day) and 19 received calcium plus vitamin D (50,000 units once or twice a week). Both groups had 50% fewer vertebral fractures than did 27 placebo-treated and 18 untreated patients.

Recommendations and safety Calcium supplementation should not be used as a substitute for sex hormone replacement, which prevents postmenopausal bone loss in most patients (55, 97, 98, 109, 112) and appears to restore intestinal calcium absorption toward normal (27). There is little justification for providing women taking estrogen replacement more dietary calcium than the RDA. It would seem prudent to advise menopausal women who are unable (for medical reasons) or who refuse to take estrogen to consume at least 1000 to 1500 mg/day of calcium in their diets with the idea that supplementation above the RDA might help prevent loss of cortical bone and the development of chronic secondary hyperparathyroidism. Similarly, elderly men (> age 60 to 65 years) could benefit from supplementation for the same reasons.

Calcium treatment is safe in the absence of conditions that cause hypercalcemia or nephrolithiasis (40). Elemental calcium intakes in excess of 3000 to 4000 mg/day should be avoided because they will cause hypercalcemia in most subjects (47).

PHOSPHORUS Increased dietary phosphorus promotes fecal calcium loss but decreases urinary calcium excretion, thus maintaining calcium balance in most normal subjects on a high phosphorus diet (38, 116). The mechanism whereby increased dietary phosphorus decreases intestinal absorption of calcium has been investigated by Portale et al (94). They showed that increasing dietary phosphorus from <500 mg/day to 3000 mg/day decreased the production rate of $1,25(\text{OH})_2\text{D}_3$ by the kidney. This observation strongly suggests that the ability to adapt to decreases or increases in dietary phosphorus depends upon the ability of the kidney to respond appropriately by increasing or decreasing $1,25(\text{OH})_2\text{D}_3$ production.

The question arises, therefore, whether increases in dietary phosphorus might have an adverse influence on calcium economy in individuals whose kidneys have a limited capacity to produce $1,25(\text{OH})_2\text{D}_3$ or in those who need to be in positive calcium balance. Portale et al (93) reported that normal dietary phosphorus levels were sufficient to suppress plasma concentrations of $1,25(\text{OH})_2\text{D}_3$ in children with moderate renal insufficiency. No studies of the influence of dietary phosphorus on calcium and bone metabolism have been reported in other populations that may be unduly sensitive to increments in dietary phosphorus above the RDA even though concern has been expressed (5, 61) that high phosphorus intakes may contribute to age-related bone loss in humans. On the other hand, chronic use of phosphate-binding antacids can

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cause phosphate depletion and skeletal demineralization (115, 117). Since antacid use is common in North America, it should be considered an important risk factor in age-related bone loss, especially in those individuals who are calcium deficient (115).

VITAMIN D Prolonged and severe deficiency of vitamin D results in osteomalacia, a disorder characterized by an increased proportion of bone matrix that is not mineralized (91). Numerous studies over the past two decades suggest that elderly persons in the United States, Israel, Great Britain, and Europe are at increased risk for developing vitamin D deficiency. The most recent studies (15, 21, 86, 88, 122) report a progressive decline with aging in serum concentrations of the major circulating form of vitamin D, 25 hydroxyvitamin D (25OHD), but little convincing evidence is presented that the incidence of osteomalacia is increased in the elderly. It is speculated that these decreased serum levels of 25OHD are due to the tendency for older persons to have less exposure to the sun (95). MacLaughlin & Holick (63) have provided data supporting an alternative and possibly complementary explanation. They found an age-dependent decrease in epidermal concentrations of 7-dehydrocholesterol (provitamin D3) and that skin biopsies from elderly subjects had as much as a twofold lower capacity to produce vitamin D3 than biopsies from young adults. It is likely, therefore, that the elderly have a decreased capacity to biosynthesize vitamin D in the skin. Gallagher et al (29) reported that a three year trial of calcitriol in osteoporotic women caused improved calcium absorption and balance and decreased urinary hydroxyproline excretion. There were also preliminary indications of a decreased rate of vertebral fractures.

PROTEIN Few epidemiologic investigations bear on the issue of whether dietary protein influences calcium economy sufficiently to be a risk factor in osteoporosis. Marsh et al (69) reported more rapid postmenopausal cortical bone loss in omnivorous compared with vegetarian women, but protein intakes and sources were not analyzed for their potential contribution to this outcome measurement. It is also possible that lifestyle differences existed between the cohorts examined that may have confounded interpretation of the results. To circumvent this latter problem, the same investigators (68) evaluated cortical bone density in adult vegetarians, using their omnivorous mates as controls; the results tended to confirm those of the previous study.

Mazess & Mather (72) reported that the bone mineral content of a group of North Alaskan Eskimos was 10% to 15% lower than values for age-matched Caucasians and that the Eskimos had a high prevalence of osteoporotic vertebral fractures. The Eskimo diet contains approximately 200–400 g of protein/day, two to four times more than in the diets of non-Eskimo Amer-

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icans; and the Ekimo diet is high in calcium and phosphorus. Clearly, dietary and lifestyle differences inherent in the Eskimo culture, and imposed by life in frigid climates with decreased exposure to ultraviolet light, could be as important or more important than protein intake in producing the bone mass changes observed in this study.

It is well established that *purified* protein, taken in increased quantities as an isolated nutrient, dramatically increases the renal excretion of calcium (41, 42). However, protein is not usually ingested as an isolated and purified nutrient; natural sources contain a myriad of other nutrients that could aggravate or counteract the calciuric effect of protein, *per se*. In 170 metabolic studies of perimenopausal women, Heaney & Recker (38) found that a 50% increase in protein intake from *natural whole foods* had a much smaller calciuric effect than that produced by purified proteins. These investigators calculated that a 50% increase in natural protein intake would lead to a negative calcium balance of -32 mg/day, an amount that approaches the -40 mg/day negative balance needed to account for the mean 1% to 1.5% loss in skeletal mass per year observed in postmenopausal women. It is thus possible that increases in dietary protein in perimenopausal women above approximately 55 grams/day could contribute significantly to the negative calcium balance frequently observed in this group. Many American perimenopausal women ingest diets containing considerably more protein than this (12). The effects of high protein diets in the elderly have not been systematically studied.

FIBER Studies over the past five decades (74, 99) have established that fiber chelates calcium (and other minerals) in the gastrointestinal tract, suggesting a potential cause of mineral deficiency. This process may increase osteoporosis risk in cultures such as in Iran where Bazari bread comprises as much as 50% of the caloric needs of children. This bread contains more calcium than white bread, but its high fiber and phytate content results in decreased intestinal absorption of calcium, magnesium, zinc, and phosphorus (99). However, the effect of fiber on mineral status at the levels consumed in the United States is unclear.

Kelsey et al (51) investigated the effects of 26 days of feeding a high fiber diet containing fruit and vegetables or a low fiber diet containing fruit and vegetable juice on calcium balance in 12 adult males. Balance was +72mg/day with the low fiber diet and -122 mg/day on the high fiber diet. In a follow-up study in which oxalate was removed from the diet, these investigators found that calcium balance was positive and was not influenced by fiber. Sandstead et al (110) found that fiber added to diets caused negative calcium balance, and they calculated that the requirement for calcium in the diet is increased as much as 150 mg/day when dietary fiber is increased by 26

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grams. Cummings et al (13) showed that the addition of 31 grams of wheat fiber to the diets of subjects already taking a high protein diet produced a greater negative balance than high protein alone, suggesting an interaction of protein and fiber.

FLUORIDE Mertz (75) has argued that fluorine is an essential trace element which is responsible for growth and maintenance of bones and teeth. However, it seems unlikely that it plays a significant role in osteoporosis risk in the majority of Americans. Usual dietary fluoride intake is only 0.3 to 0.5 mg/d (48), and there appears to be no demonstrable effect on bone structure of an additional 1 to 2 mg/d of this ion as provided in fluoridated drinking water (1 ppm) (100).

By contrast, the incidence of osteosclerosis is high in areas where the fluoride concentration in drinking water is moderately high (5 to 10 ppm) (58). Leone (57) and Bernstein et al (6) have reported a lower prevalence of osteoporosis in these areas than in "low fluoride" regions. In areas with even higher fluoride intakes, such as the Punjab in India, crippling fluorosis occurs, but asymptomatic osteosclerosis is much more common (111).

In a prospective but nonrandomized study, Riggs et al (105) reported that treatment of osteoporotic patients with pharmacologic doses of fluoride and calcium reduced the vertebral fracture rate to approximately one quarter of untreated patients and to a significantly lower number than in patients treated with calcium alone. However, recent studies by two groups (53, 101) with a randomized, placebo-controlled and double blind design showed that although trabecular bone mineral density was increased in the group given fluoride (all subjects received calcium carbonate and a moderate physical exercise program) there was no difference in vertebral fracture rate from the group given placebo. Additionally, cortical bone mineral density was decreased and the rate of nonvertebral fractures increased in the fluoride-treated group. These results suggest that an inferior quality of bone is formed in the presence of fluoride despite cotreatment with calcium.

ALCOHOL Bone formation is decreased in patients who abuse alcohol. This causes a dramatic decrease in bone mass compared with normal subjects (7, 80). Since the risk of falling is increased in alcoholics, these two factors are probably responsible for the increased risk of hip fracture in alcoholic men and women and of vertebral fractures in alcoholic men.

Animal Studies

Nordin (81) has reviewed an extensive literature describing the many species that develop decreased bone mass as a result of calcium deficiency. In all of these studies, it is clear that the bone disease produced by calcium deficiency

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most resembles osteoporosis. More recently, Jowsey & Gershon-Cohen (49) found that feeding adult cats a low calcium diet for five months decreased skeletal weight, decreased radiographic density of bone, and increased bone resorption. These changes were partially reversed by refeeding calcium. Presently, however, there is no completely satisfactory animal model of postmenopausal and/or age related osteoporosis, and this deficiency comprises a major impediment to future progress in osteoporosis research.

In contrast to the apparent inability of rather dramatic changes in dietary phosphorus to influence calcium balance in normal human subjects, there is considerable evidence in animals that diets containing relatively larger quantities of phosphorus than of calcium can cause hyperparathyroidism and bone loss (18). Almost all of these reports concern growing or aged animals and thus differ from investigations in humans (5), which in general focus on young or middle-aged adults.

ASSOCIATION OF NONDIETARY FACTORS WITH OSTEOPOROSIS

It is important for the reader to be aware that inadequate calcium nutriture *per se* is only a modest risk factor for osteoporosis. Other, nondietary factors are much more strongly associated with the disease. Risk and protective factors and their relative strengths are presented in Table 1. Several recent review articles discuss these important factors (23, 37, 60, 96).

Physical Activity

Immobilization can decrease bone mass (19, 20, 124). The osteoporosis produced can be localized (associated with fracture casting or painful limbs), generalized (associated with prolonged bed rest or space travel), or neurological (associated with paraplegia or quadriplegia). Its causes are unknown, but the absence of stress and muscle pull on bone may be a common etiologic factor. Resumption of normal weight-bearing activity restores both trabecular and cortical bone (73, 123).

The results of studies of the influence of increased physical activity on bone mass are mixed. Many studies have shown that exercise of sufficient intensity and duration as to produce amenorrhea can result in marked decreases in bone mineral density (10, 22, 59, 66, 78). However, among women who have amenorrhea from diverse causes, those who exercise regularly have greater bone density than those who are more sedentary (107).

Definite evidence is not available that regular exercise helps build peak bone mass in youth or retard bone loss in middle and old age (8). Most controlled trials have reported that moderate exercise may have a modest effect on preventing postmenopausal bone loss (2, 54, 125). Unfortunately,

Table 1 Risk and protective factors for the development of osteoporosis and fracture

Established		Suspected but not established
High	Intermediate	
Risk Factors		
Female sex	Low calcium diet	Family HX osteoporosis
Ovariectomy ^a	Early menopause	Caffeine intake
Advanced age	Thyroid Rx ^b	Alcohol, in moderation
White race	Antacids ^d	High protein diet
Thinness	Vitamin D deficiency	High fiber diet
Alcoholism	Hyperparathyroidism	Sedentary lifestyle
Steroid Rx ^b	Type I diabetes	
Disabling R. A. ^c	Cigarette smoking	
Tendency to fall		
Previous hip fracture		
Protective Factors		
Estrogens, long term use	Obesity	High calcium diet
Black race	High parity	Physical exercise
		Thiazide use

^a Bilateral ovariectomy^b Chronic glucocorticoid or thyroid hormone administration in pharmacologic doses^c Rheumatoid arthritis^d Phosphate binding antacids taken in excess

none of these latter studies used a randomized design, and sample size and statistical power was inadequate in most. The effectiveness of walking in preventing bone loss has not been well studied. Nonetheless, since moderate exercise positively affects most health conditions, it would seem prudent to advise regular exercise throughout life.

SUMMARY

Calcium requirements may vary throughout the lifespan. During the growth years and up to age 25–30, it is important to maximize dietary intake of calcium to maintain positive calcium balance and achieve peak bone mass, thereby possibly decreasing the risk of fracture when bone is subsequently lost. The RDA for age 10–25 is 1200 mg/day. Calcium intake need not be greater than 800 mg/day during the relatively short period of time between the end of bone building and the onset of bone loss (30 to 40 years old). Starting at age 40–45, both men and women lose bone slowly, but women lose bone more rapidly around the menopause and for about 10 years after. Intestinal calcium absorption and the ability to adapt to low calcium diets are impaired in many postmenopausal women and elderly persons owing to a suspected

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functional or absolute decrease in the ability of the kidney to produce $1,25(\text{OH})_2\text{D}_2$. The bones then become more and more a source of calcium to maintain critical extracellular fluid calcium levels. Available evidence suggests that the impairments of intestinal calcium absorption observed during the menopause and aging can be overcome only by inordinately large calcium intakes (1500 to 2500 mg/day). Since this amount is difficult to derive from the diet, can cause constipation, and may not prevent trabecular bone loss, it should not be used as a substitute for sex hormone replacement. Women taking estrogen replacement should be provided the RDA for calcium of 800 mg/day at a minimum. Those who cannot or will not take estrogen should be asked to ingest at least 1000 to 1500 mg/day of calcium to delay cortical bone loss and prevent secondary hyperparathyroidism. It should be emphasized that up to 2000 mg/day of calcium is safe in teenaged children and adults.

Excessive dietary intake of protein and fiber may induce significant negative calcium balance and thus increase dietary calcium requirements. It is also possible that excessive intakes of phosphate could have a deleterious effect on calcium balance in populations whose need for calcium is great (e.g. growing children) or whose ability to produce $1,25(\text{OH})_2\text{D}_3$ is impaired (e.g. the elderly). Moderation in the intake of these nutrients is urged.

Generally, the strongest risk factors for osteoporosis are uncontrollable (e.g. sex, age, and race) or less controllable (e.g. disease and medications). However, several factors such as diet, physical activity, cigarette smoking, and alcohol use are lifestyle related and can be modified to help reduce the risk of osteoporosis.

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GRAVITY, CALCIUM, AND BONE: UPDATE, 1989

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This update highlights some of the results of recent short-term flight and ground-based experiments that have contributed new insight into skeletal adaptation, calcium metabolism, and growth processes in 0 g. After 6 months in space, bone demineralization, invariably involving the os calcis (20), was found not to extend to the lumbar spine in 4 exercising cosmonauts (3). A flight experiment in the Space Shuttle crew has documented the early events in the calcium endocrine system during spaceflight (12).

On the ground, brief (<35 days) and long-term (>4 months) bed rest studies of healthy volunteers in the head-down tilt (HDT) model of weightlessness have been completed. The skeleton of the adult male responds more rapidly to unloading than previously recognized (2). Regional changes in bone density can be quantified in only 30 days, are highly individual, and follow the direction of gravitational forces in the HDT model during inactivity (1). Bone biopsy results in healthy volunteers after bed rest (11) differ from results in paraplegics from the same sampling site (21).

Flight experiments in growing rats reveal changes in the composition of bone mineral and matrix in the femur postflight that were found to be highly regional and suggestive of an effect of gravity on mineral distribution (10). These observations may be relevant to the results from an earlier Cosmos flight where artificial gravity in space was found to maintain bone strength, but not to correct the radial growth deficit (19).

Mineral in the Lumbar Spine

Ever since Krolner and Toft reported a reduction (-3.8%) in the average density of the 2nd to 4th lumbar vertebrae following therapeutic bed rest in 28 patients suffering from prolapsed intervertebral discs, there has been some concern that vertebrae in bed rest subjects and space travelers may demineralize (7). Fortunately, no significant changes were observed by Drs. Cann and Oganov, who used quantitative computer tomography to quantify the mineral content of the body of the 2nd lumbar vertebra of 4 cosmonauts before and after 6 months in space (3). These data have not completely erased the concern of osteoporosis in the lumbar spine because the Cosmonauts exercised daily.

Nevertheless, nonexercising bed rest subjects have also failed to show reduced bone density in the lumbar spine. LeBlanc et al. found no change in the density of the 2nd through 4th lumbar vertebrae in 5 of 6 subjects after 5 weeks of bed rest (horizontal); one showed a 3% decrease (8). Oganov et al. reported average increases to 12.6% in density of the spongiosa of the lumbar vertebrae of 3 bed rest subjects after 120 days in a -5° head-down tilt position (HDT) (14).

We used dual photon absorptiometry to measure the density of the 2nd through 4th lumbar vertebrae before and after 30 days HDT (-6°). Subjects were participating in a study designed to test the effect of isokinetic and isotonic exercise on orthostatic tolerance (5). Our results, shown as percent change in the histogram in Fig. 1, revealed no differences in 17 subjects, irrespective of the exercise group. Two subjects showed

changes in opposite directions (-7 and +10%), well outside the error of the test.

Calcium Endocrine System

N91-25573

To identify factors that could have contributed to the change in lumbar spine mineral in 2 of 19 subjects, we examined the diet and a variety of parameters known to influence bone metabolism. Table I enumerates the values in these two subjects before and after bed rest. Figures 2a and 2b illustrate changes in circulating hormone concentrations that may be related to the alteration in lumbar spine density. None of the values except for serum parathyroid hormone (PTH) reached values outside the normal range. Combined increases in serum PTH and weight loss (4) favored decrease in lumbar spine mineral; the opposite changes were associated with an increase. These data are consistent with the known effects of PTH to enhance bone resorption. The precise role of serum 1, 25-dihydroxyvitamin D in the change in bone mineral content is not clear.

The early response of the calcium endocrine system in 4 astronauts was documented in serum obtained before, during, and after 7 days in space on the SL2 mission (12). The published data are summarized in Fig. 3. An increase in the vitamin D hormone, 1, 25-D, within the first 36 hours of launch was the only significant change, although trends toward increases in total serum calcium and phosphorus and decreases in bioactive PTH were present in 3 astronauts. Possible explanations for the early increase in 1, 25-D include perturbations during launch, transient lack of dietary calcium associated with space motion sickness, a nonspecific stimulation of renal 1-alpha hydroxylase connected with fluid shifts, or a specific

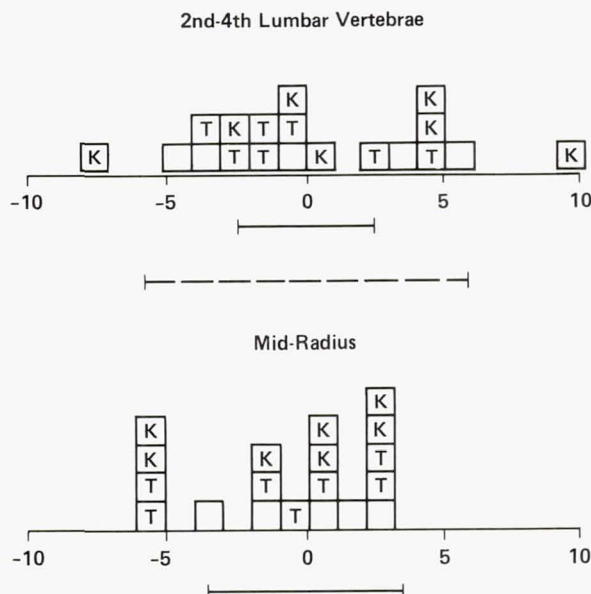


Figure 1. Percent change in density of the lumbar spine and mid-radius of 19 men referenced to each subjects basal level (0). Distribution was not affected by exercise (isotonic = T, isokinetic = K, no exercise = blank). The error of the tests are indicated by the bars, — and $2 \times \sqrt{2} \times$ coeff. of variation, by (-----).

Table I. Comparison of clinical data in two exercising subjects who showed opposite changes in lumbar spine density after 30 days head-down tilt bed rest.

Study day, from 1st bed rest day	A			B		
	-5	4	27	-5	4	27
Age, years	42			37		
Weight, kg	68		68.6	83.8		82
Height, cm	171			183		
Plasma volume, ^a ml/kg	46.3		40.5	48.2		39.9
Body fat, ^a %	7.7			8.0		
Serum						
Total calcium, mg/dl	9.1		8.8	9.9		9.8
Ionized calcium, mg/dl	4.20		5.00	4.88		4.96
Total protein, g/dl	7.2		7.0	7.2		7.2
Phosphorus, mg/dl	2.0		3.2	2.3		2.3
Parathyroid hormone, ^b pg/ml	20	24	17	24	59*	44
1, 25-dihydroxyvitamin D, pg/ml	16	17	37	33	28	30
Cortisol, ^c ug/dl	16.9		15.6	9.5		11.3
Testosterone, ^c total, ng/ml	882			871		
Testosterone, ^c free, ng/dl	25			22.2		
Urine						
Creatinine clearance, ml/min/1.73m ²	128		123	108		118
Calcium, mg/24 hr	229		165	182		301
Hydroxyproline, mg/24 hr	21		15	36		42
Diet during study ^d						
Calories, kCal/kg	42		45	34		36.8
Calcium, mg	1281		1398	1274		1431
Phosphorus, mg	1816		1959	1883		2020
Sodium, mg	5756		5941	5615		5976
Protein, g	117		119	114		119
Bone density ^e						
Radius, gm/cm	1.304		1.337	1.422		1.434
Lumbar spine, L2-4, gm/cm ²	1.175		1.293	1.875		1.725

Analysis of ^a by J. Greenleaf, ^b by R. Marcus, ^c by C. Wade, ^d by R. Williams, and ^e by M. Powell.

*Above the normal range.

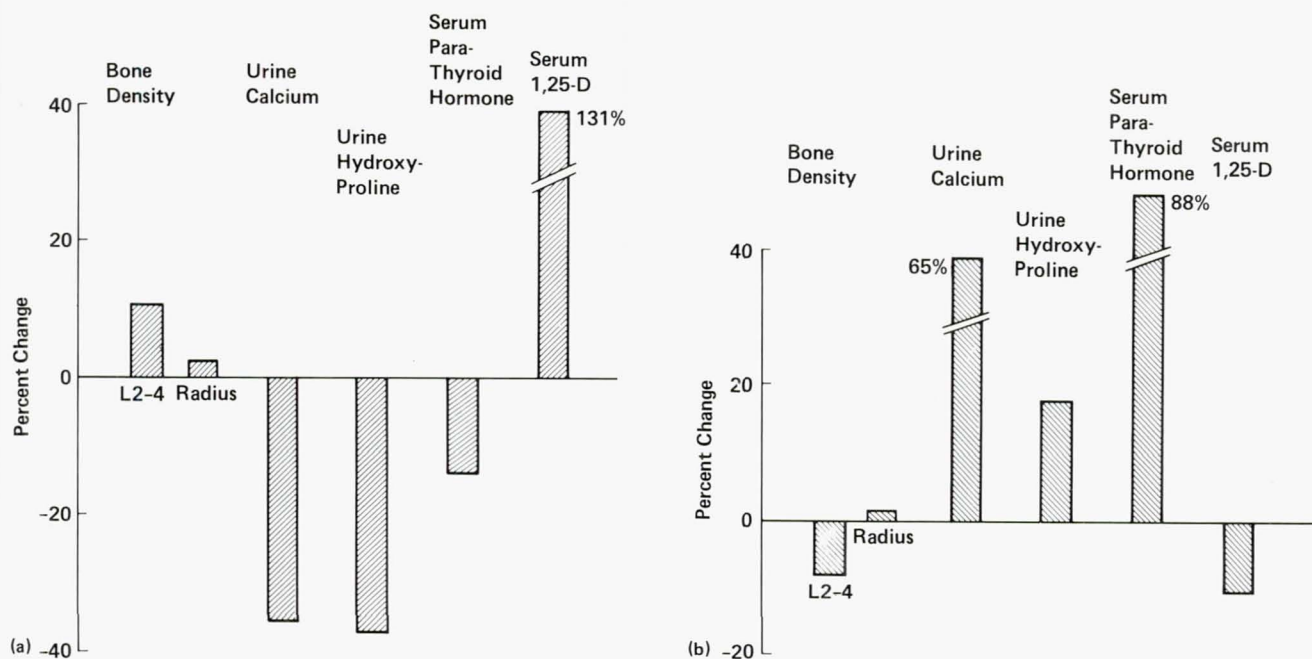


Figure 2. Changes in some parameters of calcium homeostasis, referenced to pre-bed rest levels, in subject A (a) who showed an increase, and subject B (b) who showed a decrease in lumbar spine density after 30 days bed rest. Of interest, both subjects performed isokinetic exercise for 30 min twice daily (5).

SPACE FLIGHT

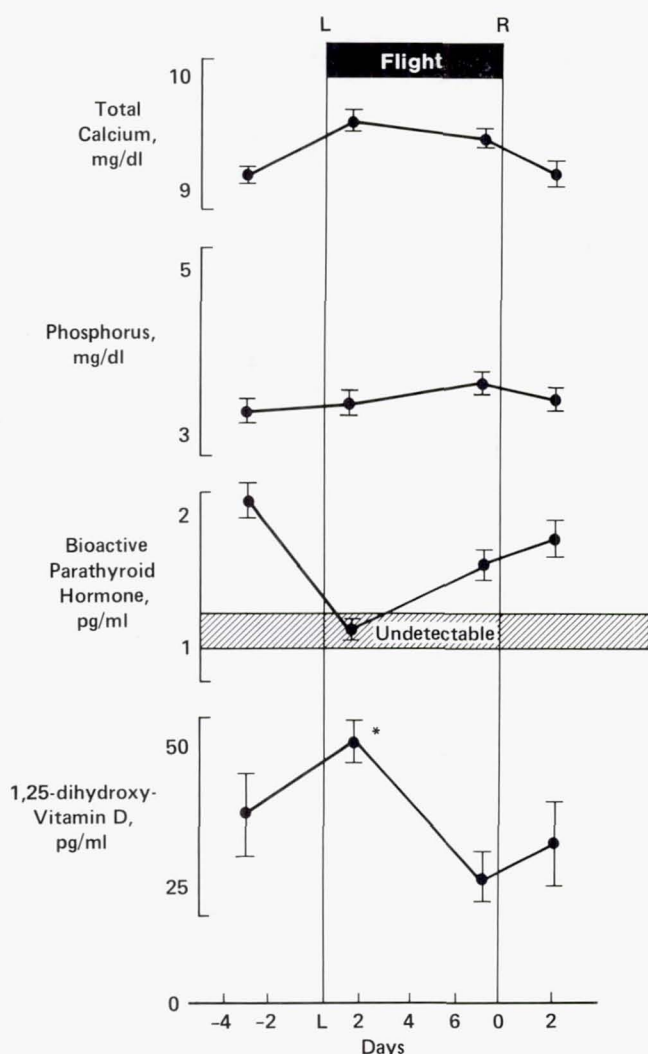


Figure 3. Mean values (\pm SE) in the serum of four astronauts obtained 1 week before, during, and the first week after launch (L) of a 7-day shuttle spaceflight (SL2) (data replotted from reference 13).

response in vitamin D metabolism to a change in a biomechanical stimulus originating in bone or muscle. Differences in the values during the first 24 hours did not seem to affect 7-day values, which are in the direction of being lower, but are not different from preflight values. The important contribution of these few samples taken during a flight is the preliminary knowledge that biologically active PTH, undetectable in serum after 36 hours in space, was not increased after 7 days, nor was 1, 25-D. While excesses of serum PTH cannot be responsible for the early mobilization of bone calcium, transient increase in 1, 25-D may be.

The above short-term data in flight differs from the results of a 7-day HDT study conducted at Ames Research Center, in which no changes were found during the first 36 hours. However, after 7 days, the trends to lower serum PTH and 1, 25-D in flight and on the ground were similar (11). The long-term Soviet bed rest study shows changes compatible with parathyroid hyperplasia with increases in serum calcium

and PTH (especially after 49 days), suggestive of differences in early and late responses in the calcium endocrine system (13).

Of interest, in the Soviet bed rest study, were early increases in serum levels of calcitonin, an inhibitor of bone resorption, that gradually decreased to lower than basal levels after 3 months. Given the variations in both assay methods and bed rest protocols, the status of the calcium endocrine system, at least, after the first week in space or bed rest in healthy individuals, remains uncertain.

Bone Morphology

If newer concepts in the role of PTH and 1, 25-D in the processes of bone remodeling are correct, i.e., that PTH governs the differentiation and number of bone cells, and 1, 25-D, cell activity (9), the pattern of circulating hormone levels from the SL2 mission suggests the following early sequence of events: enhanced mobilization of calcium from bone related to the increase in 1, 25-D followed by suppressed mineralization in unloaded bones after a few days, with no increase in the number of osteoclasts or osteoblasts. Standard post mortem examination of some of the bones of 3 Cosmonauts after 28 days in space showed normal histology, fewer vascular channels than a control sample, and some increase in the porosity of the femoral epiphysis and diaphysis, but not in the rib, vertebrae, or calcaneus (16). Jowsey's analysis of the iliac crest of patients after 4-17 days horizontal bed rest for conditions unrelated to the skeleton, demonstrated reduced bone formation and no difference in the extent of resorption surfaces from normal in 11 of 14 patients. Cell counts are not in the report (6).

Following a 4-month period of bed rest in 3 healthy Soviet volunteers, Vico et al. found a two-fold increase in resorption surfaces, no increase in cell number, and reduced bone formation rate in specimens from the iliac crest (21). A puzzling observation was no measurable change in the volume of bone in healthy bed rest subjects, unlike patients with paraplegia (11). That the normal subject shows changes in surface morphology indicative of bone loss with no apparent diminution in volume at the two-dimensional level, suggests some form of compensation in microarchitecture. Either standard measurements may not be sensitive enough to detect losses in volume or other measurements involving the three-dimensional structure of bone, not usually done, may be needed to show how normal subjects maintain bone volume.

Gravity-Dependent Gradients of Mineralization

Comparison of the increments in whole-body calcium of rats exposed to 0, 1, and 2 g reveals accumulation of bone mineral directly related to the gravitational force (15). The mechanism of this acquisition of skeletal mineral must involve systemic as well as local processes. The cardiovascular system, whose general structure is oriented in the direction of gravity and where blood vessels, flow, and volume are known to differ at the local bone level in active and inactive individuals, is the most obvious candidate to influence bone mass.

Until recently, however, there were no data that suggested that there was a generalized cardiovascular effect on bone or that a shift of the hydrostatic column of pressure with changes in position, was associated with changes in bone mineral. In the tail-suspended rat, Roer and Dillman found the expected decrease in ash in the bones of the unloaded lower extremities, no change in the humerus and ulna, and

importantly, an increase in bone ash in the skull (17). By dual photon absorptiometry, the density of the head region of adult bed rest subjects was found to be increased an average of 10% after a 30-day HDT study (1). These two studies suggest a gravity-dependent distribution of mineral in the whole skeleton, which may be a function of changing pressures, fluid flow, or volume in the cardiovascular system in response to change in position.

During the Cosmos 936 mission, centrifugation in orbit permitted comparison of the effects of gravity on the strength and growth of the femur of young rats in space (19). Rats treated with artificial gravity showed the same increases in density and strength during the 18.5-day flight as ground controls; however, the growth defect was not improved. Spengler et al. attributed the growth deficit to poor adaptation of the rats to the short-arm radius centrifuge and concluded that centrifugation normalized material properties, i.e., quality, but not the quantity of the femur. These paradoxical findings following artificial gravity could be explained by the recently observed linear gradients of mineralization in the diaphysis of the femur (10). At 1 g bone mineral concentration was lower in the distal than in the proximal diaphysis of the femur of the 14-week old rat, a disparity that persists in flight, but tends to disappear by 16 weeks on Earth. Because of the logistical problems connected with the 1887 flight where these diaphyseal gradients of mineralization were observed, and because the results differ from our expectation that mineral deposition proceeds from the center of a growing bone proximally and distally, confirmation of this observation is needed. Collectively, all of the above studies reveal an important connecting link between gravity, per se, and bone mineral distribution and deposition, most likely related to the cardiovascular system. The interaction of what appear to be gravity-dependent gradients visible at the whole-body and organ level, with the highly regulated processes that change bone structure at the local tissue level in response to biomechanical forces is not now apparent.

In summary: Advances in recent years have enabled us to recognize that two principal components of calcium metabolism, the calcium endocrine system and bone, respond promptly (within days), to changes in body position and weightlessness. The vitamin D hormone may be the best candidate for mobilizing bone mineral early, and newly identified gravity-dependent gradients, probably involving the cardiovascular system, may have a significant role in its distribution at the whole-body level. These observations have given us a new perspective on the results of balance studies in healthy subjects and astronauts (18,22). During inactivity or weightlessness, negative balances in bone minerals may be more directly a reflection of diets, and alterations in the function of the gastrointestinal tract and kidney that parallel, but do not necessarily derive from the highly localized activities concerned with the restructuring of bone and redistribution of bone mineral to meet new functional requirements. These studies imply that bone biomechanics are more severely affected by spaceflight than bone mass.

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Orthostatic Hypotension

C. GUNNAR BLOMQVIST

Orthostatic hypotension is a common and sometimes disabling condition. Likely causes include many different defects that singly or in combination affect major mechanisms controlling blood flow, vascular resistance, arterial pressure, and intravascular volume. The control systems are complex, and their interactions are poorly understood. As a consequence, obvious and straightforward therapeutic approaches sometimes prove ineffective, but seemingly paradoxical measures are often helpful. These characteristics combine to make orthostatic hypotension a challenging topic.

CAUSES OF RECURRENT EPISODIC ARTERIAL HYPOTENSION

Syncope is a common manifestation of orthostatic hypotension. The principal mechanism of syncope, including the orthostatic variety, is a transient reduction in cerebral blood flow. Causes of recurrent episodes of arterial hypotension include cardiac *dysrhythmias*. Bradyarrhythmias, tachyarrhythmias, and intermittent atrioventricular conduction blocks can cause reductions in cardiac output of sufficient magnitude to impair cerebral perfusion, particularly in patients with coexisting cerebrovascular disease. *Mechanical obstruction* of systemic or pulmonary blood flow may produce global cerebral ischemia with syncope. Such conditions include valvular aortic or pulmonary stenosis, idiopathic hypertrophic subaortic stenosis, atrial myxoma, and pulmonary embolic or vascular disease with pulmonary hypertension.

A wide range of emotional and somatic afferent stimuli can precipitate *vasodepressor* or *vasovagal syncope*. Neither term is strictly accurate. The cardiovascular response usually includes both bradycardia and vasodilatation, and impulse flow is altered in both the parasympathetic and sympathetic portions of the autonomic nervous system. The typical psychological circumstances involve a perception of an actual or symbolic injury that the victim feels that he or she should be able to face without fear. Obligations to submit to painful or unfamiliar diagnostic or therapeutic procedures

are prime examples.¹ Among the somatic mechanisms are carotid sinus hypersensitivity² and abnormal impact of afferent impulses from the ear, mouth, larynx, and pharynx (*e.g.*, in glossopharyngeal neuralgia).³ Simple swallowing (deglutition syncope)⁴ may precipitate vasodepressor syncope in some persons. The hemodynamic events have been well documented.^{1,5-7} A typical sequence includes an initial phase with moderate tachycardia followed by a marked fall in heart rate and arterial pressure. The depressor phase of the response has many features in common with orthostatic hypotension that progresses to syncope and later will be discussed in some detail.

PATHOPHYSIOLOGY OF ORTHOSTATIC HYPOTENSION

PRINCIPAL FEATURES

Orthostatic or postural hypotension may be defined as the inability to maintain adequate arterial pressure and tissue perfusion in the upright position. The brain is almost always the organ most vulnerable to postural hemodynamic changes, but orthostatic angina pectoris has been described.⁸ Syncope is the manifestation of grossly inadequate cerebral blood flow. Lesser degrees of hypoperfusion cause vague weakness and postural dizziness or faintness. Many different clinical conditions are associated with orthostatic intolerance. Some patients have severe and widespread structural neurologic and cardiovascular abnormalities. Others appear to have strictly functional disorders.

Two major mechanisms cause orthostatic intolerance: (1) relative central hypovolemia with postural decreases in cardiac filling and stroke volume to subnormal levels and (2) inadequate regulatory responses to the decrease in stroke volume and cardiac output.

The conventional terminology in this area is often inappropriate and confusing because it is based exclusively on the responses mediated by the sympathetic nervous system. It would be preferable to use dual descriptors referring to changes in intravascular volume and to regulatory responses. "Sympatricotonic orthostatic hypotension" may then be characterized as hypovolemic hyperreactive orthostatic hypotension. The "asympatricotonic" variety would be referred to as normovolemic hyporeactive orthostatic hypotension.

GRAVITY, CARDIOVASCULAR PRESSURE-VOLUME RELATIONSHIPS, AND STARLING'S LAW

All intravascular pressures have a gravity-dependent hydrostatic component (Fig. 1).^{9,10} The interactions between the gravitational field, the position of the body, and the structural and functional characteristics of the blood vessels determine the distribution of intravascular volume. This, in turn, has major effects on cardiac filling and pump function.

Data on human blood volume, its distribution, and vascular pressure-volume relationships have been reviewed by Blomqvist and Stone.¹⁰ Total blood volume in mammals is a linear function of body weight. Mean values in normal adult humans cluster around 75

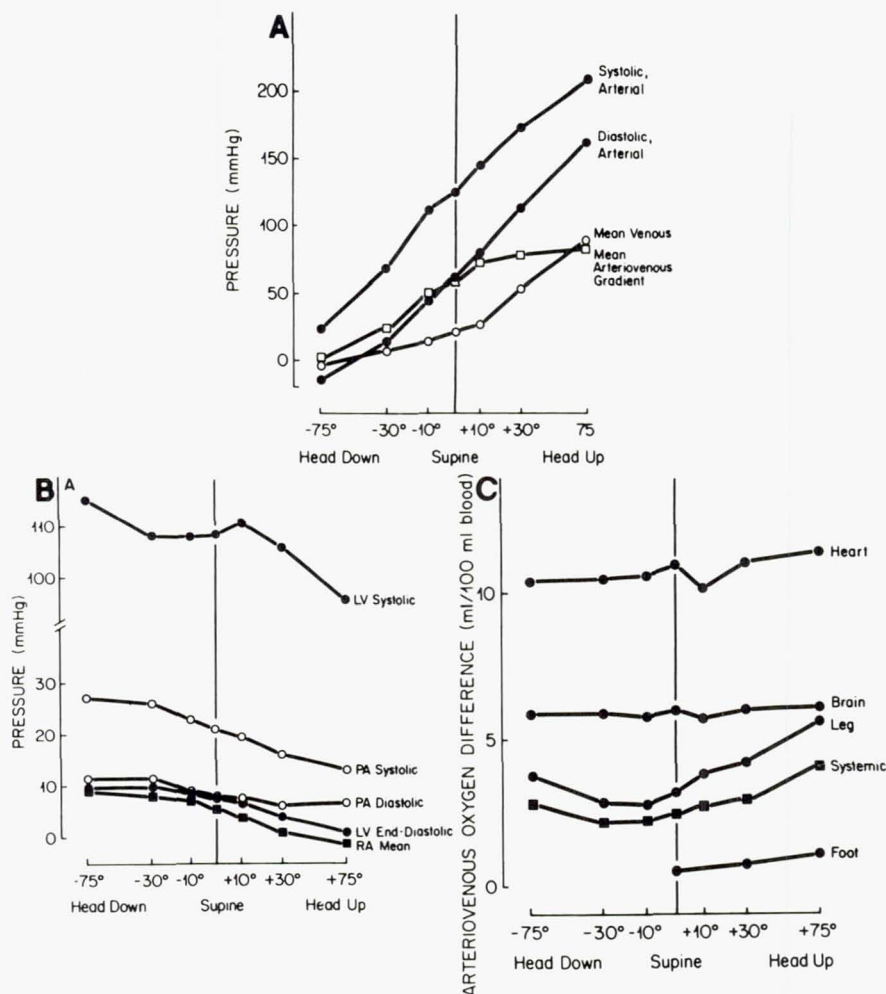


Fig. 1. Responses to graded head-up tilt in ten young normal men. Intravascular pressures in foot (A) and in central circulation (B). Arteriovenous oxygen difference (C). Angle of tilt (horizontal axis) plotted as sine function to provide linear scale for primary hydrostatic effects of body-position changes, based on data from Katkov and Chestukhin.⁹ (LV, left ventricle; PA, pulmonary artery; RA, right atrium) (Blomqvist CG, Stone HL: Cardiovascular adjustments to gravitational stress. In Shepherd JT, Abboud FM (eds): Handbook of Physiology, Section 2, The Cardiovascular System. Volume III: Peripheral Circulation and Organ Blood Flow, Part 2, pp 1025-1063. Bethesda, MD, American Physiological Society, 1983)

ml/kg, corresponding to a total of 5 to 5.5 liters in a 70-kg person. High levels of physical activity and adaptation to a hot climate cause expansion of the blood volume with balanced increases in red blood cell mass and plasma volume.

Approximately 70% of the total blood volume is contained in the systemic veins; the heart and the lungs account for 15%, the systemic arteries for 10%, and the capillaries for 5%. Effective total vascular compliance represents the summed compliances of the various vascular compartments. It is dominated by the systemic veins. Measurements are derived by monitoring central venous pressure during acute changes in blood volume. Normal human compliance values are of the order of 2 to 3 ml/mm Hg/kg. Effective compliance is an empiric measurement, complicated by reflex hemodynamic adjustments with secondary redistribution of venous volume, by delayed compliance (viscoelastic creep of the vessel walls), and by adjustments in plasma volume by tissue filtration. Nevertheless, it provides a useful measure of the impact on right-sided cardiac filling pressures of acute hypovolemia and hypervolemia.

A simple Frank-Starling relationship (stroke volume as a function of end-diastolic volume or pressure) is a

reasonably accurate descriptor of cardiac performance during postural changes in healthy persons at rest. There are normally no major changes in arterial blood pressure. Afterload, expressed as end-systolic wall stress, is usually slightly reduced in the upright position. The normal left ventricle ejects more than half of its end-diastolic volume, usually between two thirds and three fourths (Table 1).^{11,12}

Stroke volume varies in direct proportion to changes in end-diastolic volume (Fig. 2).¹³ Increases in ejection fraction with secondary increases in stroke volume, mediated by positive inotropism, are of only minor functional significance during acute interventions that primarily affect ventricular filling. Arterial pressure is maintained by adjustments in heart rate and systemic vascular resistance.

CEREBRAL PERFUSION

Cerebral blood flow is normally tightly controlled by autoregulation. It remains stable over a wide range of mean arterial pressure at normal levels of arterial carbon dioxide partial pressure. Cerebral blood flow usually starts to decrease significantly when driving pressure (mean arterial pressure at the eye level) falls

TABLE 1. Postural Cardiovascular Adjustments in Normal Human Subjects

Parameter	Supine	Sitting	p
Left ventricular volume (ml)*			
End-diastolic	107 ± 10	85 ± 6	<0.02
Endsystolic	34 ± 4	32 ± 5	
Stroke	76 ± 8	55 ± 5	<0.05
Ejection fraction (%)	76 ± 2	72 ± 4	
Heart rate (beats per minute)†	73 ± 4	84 ± 4	<0.001
Pressure (mm Hg)			
Brachial artery	96 ± 3	99 ± 4	
Systolic	130 ± 5	132 ± 5	
Diastolic	76 ± 3	82 ± 3	<0.05
Pulmonary artery	13 ± 1	13 ± 1	
Pulmonary capillary wedge	6 ± 1	4 ± 1	<0.001
Left ventricular end-diastolic	8 ± 1	4 ± 1	<0.001
Stroke index (ml/m ²)	50 ± 5	35 ± 3	<0.001
Cardiac index (liters/min/m ²)	3.5 ± 0.3	2.8 ± 0.2	<0.001

* Left ventricular scintigraphic data (mean ± standard error) from seven young normal subjects studied by Poliner and co-workers.¹¹
† Hemodynamic measurements from ten sedentary men, aged 32 to 58 examined by Thadani and Parker.¹²

below 50 mm Hg. Consciousness may be lost when blood flow falls below one fourth of normal, which usually occurs at a mean pressure of about 40 mm Hg.¹⁴ The hydrostatic gradient between the levels of the heart and the brain in the upright position adds 30 mm Hg to the required pressure as measured at the heart level. A mean arterial threshold pressure of 70 mm Hg corresponds to systolic and diastolic pressures of about 80/65 mm Hg. A significant shift of the autoregulatory range to the left is likely to occur in chronic autonomic dysfunction¹⁵ with orthostatic hypotension, and a right-ward shift is a feature of systemic hypertension.

NORMAL RESPONSES TO ORTHOSTATIC STRESS

A change in body position from supine to standing or sitting initiates a well-defined sequence of events^{10,16,17}:

1. Blood volume is redistributed away from the heart. About 500 ml is removed from the intrathoracic region to the legs. An additional volume of 200 to 300 ml is transferred to the veins in the buttocks and the pelvic area.
2. Cardiac filling pressures fall, and stroke volume decreases, usually by 20% to 30%.
3. An equally large acute decrease in arterial pressure is prevented by rapid baroreflex-induced increases in heart rate and systemic vascular resistance. Additional neurohumoral mechanisms are activated within minutes to preserve adequate intravascular volume and to help maintain arterial pressure.
4. Cerebral perfusion pressure is kept within the autoregulatory range.

The principal features of the human cardiovascular response to orthostatic stress are shown in Figure 3.

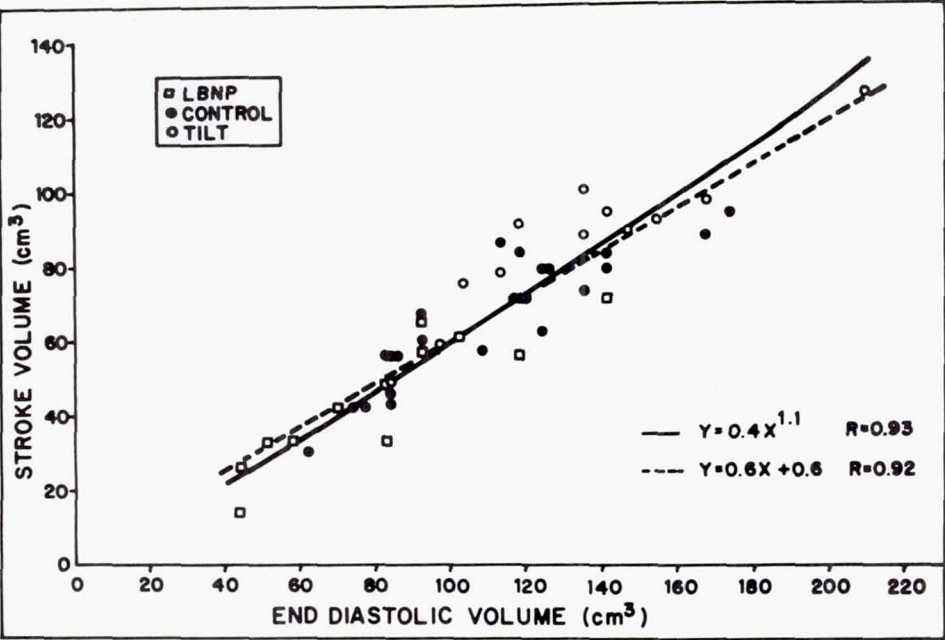


Fig. 2. Relationship between left ventricular stroke volume and end-diastolic volume. Echocardiographic measurements in 12 normal young men. Large variations in preload were introduced by head-down tilt at 5° and lower body negative pressure (LBNP) at -40 mm Hg. (Nixon JV, Murray RG, Leonard PP et al: Effects of large variations in preload on left ventricular characteristics in normal subjects. Circulation 65:698-703, 1982) By permission of the American Heart Association, Inc.

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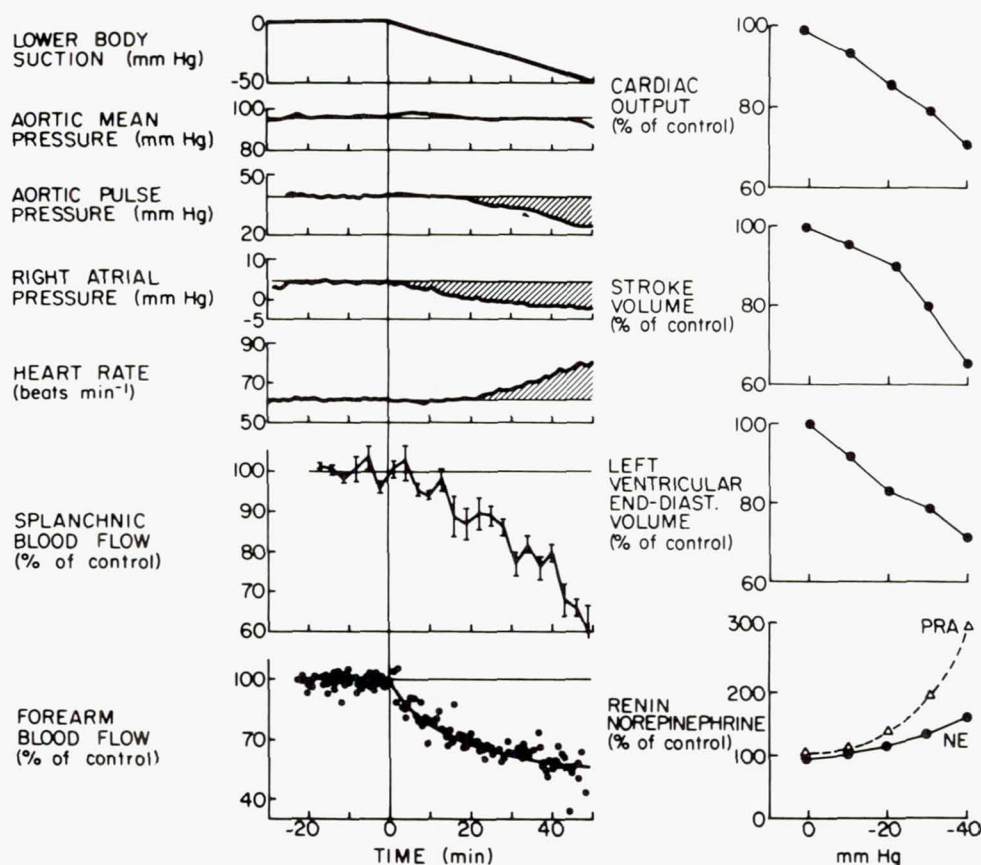


Fig. 3. Cardiovascular responses to graded lower body negative pressure. Panels on the left show average responses to suction applied at a continuous rate of $-1 \text{ mm Hg min}^{-1}$ for 50 minutes.¹⁸ Panels on the right show central circulatory responses to 10-mm Hg steps in negative pressure down to -40 mm Hg .¹⁹ (Rowell LB: Human Circulation: Regulation During Physical Stress, pp 137-173. New York, Oxford University Press, 1986)

The data represented in the figure were collected during lower body negative pressure (LBNP). Application of LBNP produces a redistribution of intravascular volume similar to that which occurs during a transition from supine to sitting or standing. The use of LBNP facilitates many measurements. LBNP also gives the experimenter better control of the stimulus by minimizing skeletal muscle activity that has major effects on the blood volume distribution and on the dynamic cardiovascular response. Furthermore, in the microgravity environment of space, LBNP provides a means of studying the equivalent of gravitational postural shifts of intravascular volume.

Figure 3 shows a progressive decrease in right atrial pressure, left ventricular end-diastolic volume, stroke volume, and cardiac output. Aortic pressure during the early stages is maintained by vasoconstriction only. Initially this involves the skin and skeletal muscle (forearm), but later the splanchnic region is also involved. Further decreases in stroke volume are partially offset by increasing heart rate. Plasma levels of norepinephrine increase, representing overflow from vascular receptors, and plasma renin activity levels are also elevated in response to large decreases in cardiac filling.¹⁷⁻¹⁹

TOTAL BLOOD VOLUME AND MECHANISMS CONTROLLING ITS DISTRIBUTION

Variations in total blood volume well within the physiological range may affect orthostatic tolerance.^{20,21} The relative degree of peripheral pooling is also important. Patients with massive venous varicosities or a congenital absence of the venous valves have postural hypotension and decreased exercise capacity in the upright position.²² Ambient temperature also affects the degree of peripheral pooling, probably mainly by altering skeletal muscle tone. Heat markedly reduces, and cold increases, orthostatic tolerance.²³ Relative rather than absolute magnitude determines the hemodynamic impact of peripheral redistribution of blood. Subsets of patients (*e.g.*, those with mitral valve prolapse syndrome) with orthostatic hypotension and reduced total blood volume may pool no more or even less than normal controls in terms of absolute volume.²⁴ Other patients with intact autonomic function have a combination of increased absolute peripheral venous pooling and reduced total blood volume.²⁵

Considerable controversy exists regarding the extent to which active reflex-mediated venomotor changes contribute to cardiovascular homeostasis during

changes in posture.^{17,26,27} In general, active venoconstriction may occur in the skin and in the splanchnic region. Veins supplying skeletal muscle are poorly innervated, and plasma concentrations of norepinephrine rarely reach levels that would produce venoconstriction. Furthermore, the deep veins in the leg have very thin walls. Venous compliance is largely determined by the characteristics of skeletal muscle. Mayerson and Burch measured intramuscular pressures in young persons who had had multiple episodes of orthostatic hypotension progressing to syncope.²⁸ Fainters had lower intramuscular pressures in the leg at rest and subnormal pressure increases during head-up tilt. Buckey and associates used a combination of magnetic resonance imaging (MRI) and occlusion plethysmography to examine the capacity of the deep leg veins.²⁹ At distending pressures equivalent to the hydrostatic venous pressures in the upright position, more than one half of the increase in leg volume was accommodated by the deep veins (Fig. 4). This finding implies that the properties of skeletal muscle are likely to affect significantly the distribution of venous volume and cardiac filling also at rest when the muscle pump is inactive.

Local reflex mechanisms may contribute to the vascular response to orthostatic stress. In experimental animals, activation of venous afferent fibers by distention produces reflex-induced leg muscle activity that may counteract postural pooling.^{30,31} However, attempts to demonstrate a similar reflex in humans performed by Blomqvist and associates have been unsuccessful. Vasoconstriction with decreased limb blood flow in response to local venous distention mediated by a local (axonal) sympathetic reflex mechanism has been demonstrated by Henriksen and Sejrsen.^{32,33}

CARDIAC PRESSURE-VOLUME CHARACTERISTICS

Cardiac pressure-volume characteristics during diastole (Fig. 5) are likely to modulate the systemic effects of any given decrease in intrathoracic blood volume. In the supine position in normal sedentary subjects, the left ventricle appears to be operating close to its maximal functional diastolic volume. Increases in filling pressure during exercise or intravenous fluid loading, or during the two interventions combined, produce only minor increases in end-diastolic volume and stroke volume.^{10,34,35}

Hypovolemia decreases orthostatic tolerance for several different reasons. Large losses of intravascular volume lower supine filling pressure, end-diastolic volume, and stroke volume and magnify the orthostatic decreases in ventricular filling and stroke volume. Any absolute amount of postural venous pooling will represent a larger relative peripheral transfer in the hypovolemic subject. More importantly, hypovolemia alters the effective ventricular diastolic pressure-volume characteristics. The normal pressure-volume curve is nonlinear with a larger change in volume for any change in pressure at low filling pressures.³⁶ Hypovolemia causes a leftward displacement of the operating

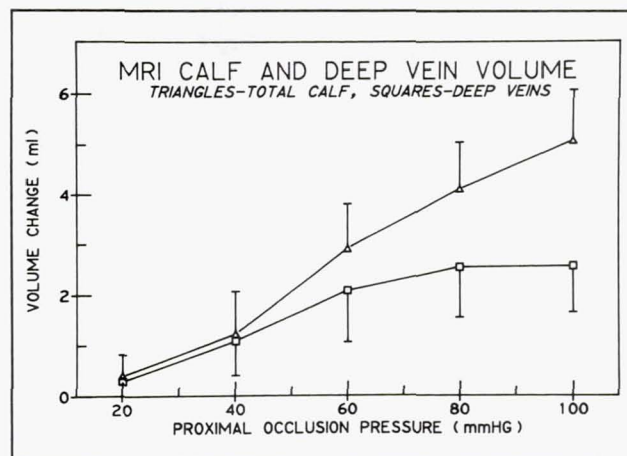


Fig. 4. Changes in deep venous volume and total leg volume with increasing venous occlusion pressures. Measurements derived by quantitative analysis of cross-sectional magnetic resonance images of the lower leg. (Buckey JC, Peshock RM, Blomqvist CG: Deep venous contribution to hydrostatic blood volume change in the leg. *Am J Cardiol* 62:449-453, 1988)

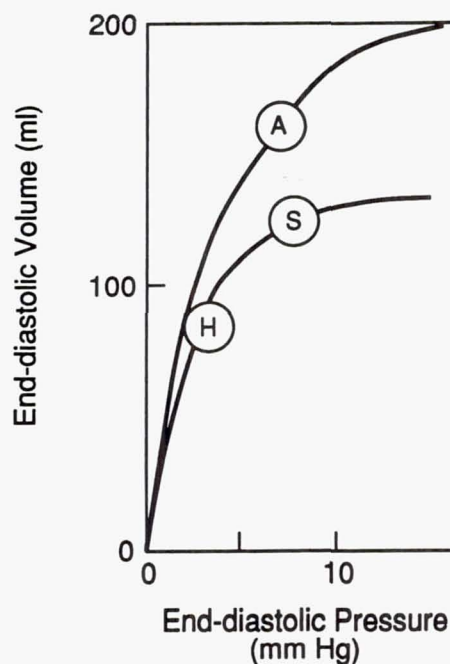


Fig. 5. Potential mechanisms by which the diastolic pressure-volume characteristics of the normal left ventricle may affect orthostatic tolerance. In the supine position, sedentary subjects (S) operate at near-maximal volume, that is, on the relatively flat portion of the curve. Hypovolemia with a decrease in filling pressure (H) will cause a shift toward the steep portion and potentiates the effects on ventricular volume of further decreases in filling pressure. There is suggestive evidence that endurance athletes (who usually have large diastolic volumes) normally operate on the steep portion of the function curve (A). This provides a mechanism augmenting end-diastolic volume and stroke volume when filling pressures increase during exercise. However, there will also be a large orthostatic decrease in stroke volume that may help explain why high levels of aerobic fitness sometimes are associated with orthostatic hypotension. (Based in part, on data from Parmley WW: Ventricular function. In Parmley WW, Chatterjee K (eds): *Cardiology*, vol 1, chap 5. Philadelphia, JB Lippincott, 1988)

point away from the flat portion of the function curve (where moderate increases and decreases in filling pressure have little effect on end-diastolic volume and stroke volume) toward the steep portion of the curve where any further reduction in filling pressure will cause a large decrease in stroke volume.

NEUROHUMORAL REGULATION

Short-term regulation of arterial blood pressure is accomplished mainly by neural mechanisms. Carotid, aortic, and cardiopulmonary mechanoreceptors are involved. These receptors all respond to deformation, that is, to stretch or compression caused by increased intracavitary or transmural pressures. Cardiopulmonary receptor densities are particularly high at the left-sided atriovenous junctions and in the inferoposterior portion of the left ventricular wall. Afferent impulses travel with the vagus and the glossopharyngeal nerves. The nucleus of the tractus solitarius is the primary site of interaction between impulse traffic in the baroreceptor pathways and activity within the central nervous system.³⁷ Efferent fibers reach the sinus and atrioventricular nodes, the cardiac ventricles, and the systemic arterioles and veins by vagal and spinal cord pathways.

A fall in intravascular or intracardiac pressure decreases afferent impulse traffic. This releases central inhibitory activity and alters the efferent impulse flow. Parasympathetic drive decreases, but α - and β -adrenergic activities increase. Responses of the target organs include increased heart rate, increased contractility, and vasoconstriction with reduced blood flow to the skin, to inactive skeletal muscle, and to the renal and splanchnic regions. The majority of the β -receptors innervated by the sympathetic nerves are of the β_1 subtype. They regulate heart rate, cardiac contractile state, and release of renin from juxtaglomerular cells. The β_2 -receptors of the resistance vessels in skeletal muscle have a vasodilator function but are not innervated.³⁸

The existence of a triplicate system for neural control of blood pressure is well established,^{39,40-42} but the interactions and degree of functional overlap between the three principal baroreflexes (carotid, aortic, and cardiopulmonary) are still poorly understood. Data from experiments in nonhuman species are not necessarily applicable to human physiology and medicine. Distributions of hydrostatic gradients and regional blood volume are markedly different in humans and quadrupeds, but interesting, minimally invasive and safe techniques have been developed for human use in the study of specific aspects of short-term reflex regulation of arterial pressure.

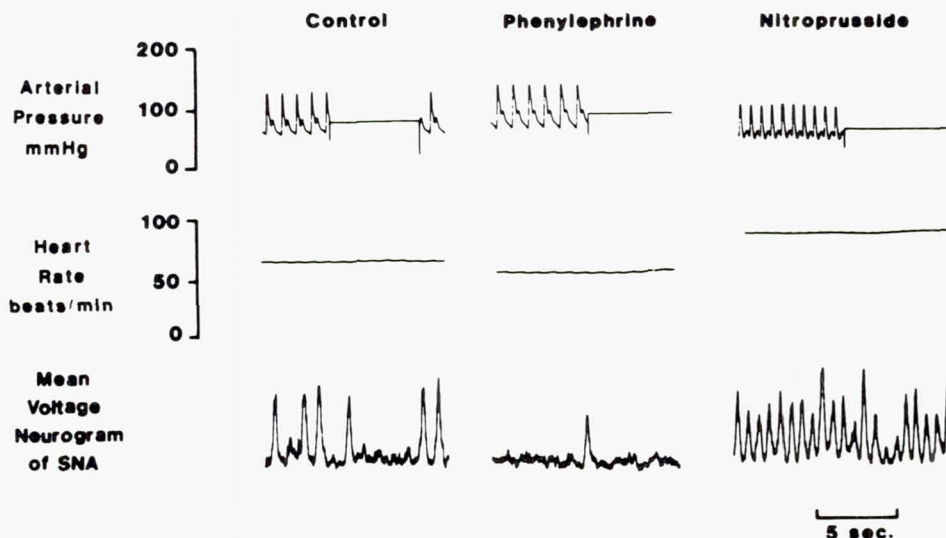
DIRECT MICRONEUROGRAPHIC STUDIES OF MUSCLE SYMPATHETIC NERVE ACTIVITY

A microneurographic technique for direct recording of human sympathetic nerve activity has been developed by Hagbarth and Vallbo⁴³ and has been applied extensively to the study of cardiovascular physiology by Wallin⁴⁴⁻⁴⁶ and others.⁴⁷⁻⁵¹ The peroneal and median nerve are relatively easily accessible. A thin tungsten electrode is inserted into a nerve fascicle supplying either muscle or skin. An impulse pattern with pulse-synchronous bursts in response to changes in blood pressure identifies a muscle nerve supplying vascular terminals (Fig. 6). Quantitation of the impulse traffic provides a direct measure of efferent vasoconstrictor activity. The time resolution is excellent, and measurements are highly reproducible in a given subject.

CAROTID AND AORTIC BARORECEPTORS

More than 30 years ago, two British flight surgeons, Ernsting and Parry, described an ingenious noninvasive technique to test carotid baroreceptor function.⁵² Suction applied to the neck area by means of an airtight collar produces an increase in transmural arterial pressure and increased deformation of the mechano-

Fig. 6. Arterial baroreceptor reflex responses: effect of elevating phasic and mean arterial pressure with phenylephrine and lowering pressure with nitroprusside on heart rate and efferent muscle sympathetic nerve activity (SNA) in a normal subject. SNA is pulse synchronous. An 8-mm Hg increase in arterial pressure (phenylephrine) caused marked reflex inhibition of SNA and a reflex fall in heart rate. A 15-mm Hg fall in arterial pressure (nitroprusside) caused an increase in SNA and heart rate. (Aksamit TR, Floras JS, Victor RG et al: Paroxysmal hypertension due to sinoaortic baroreceptor denervation in humans. Hypertension 9:309-314, 1987)



ceptors. The stimulus closely simulates an increase in intravascular carotid pressure, but there are no significant direct hemodynamic effects.

The approach has been refined and used extensively by Eckberg and his associates to evaluate the vagally mediated effects on heart rate.⁵³⁻⁵⁷ A computer-controlled system delivers an electrocardiogram-triggered ramp of neck collar pressures. Each pressure level is imposed only during a single cardiac cycle. The reflex response time is very short. The effect of a change in transmural pressure is measured during the next cardiac cycle. The pressure ramp is easily repeated and stimulus-response curves (Fig. 7) can be based on multiple measurements. Characteristic abnormalities have been described in hypertension.⁵⁵ The operating point is reset in mild disease, and the sensitivity or slope is reduced in more advanced cases.

Major assets of this approach are the lack of effect on the native hemodynamic state and the relative ease by which complex quantitative data can be acquired. On the other hand, the procedure generates data only on the heart rate component of the reflex. Activation of the carotid baroreceptors by increased transmural pressure of longer duration also affects the sympathetic nerve traffic to the resistance vessels in skeletal muscle.⁵¹ At least theoretically, carotid baroreceptor function may be normal in the presence of attenuated heart rate responses if the vasomotor effects are enhanced.

The operating characteristics of the carotid and aortic baroreflexes appear to be different in different species. In dogs, the aortic reflex has a higher threshold and lower sensitivity than the carotid baroreflex. Ferguson and associates⁵⁸ and Sanders and co-workers⁵⁹ used a combination of the direct sympathetic nerve recording technique and the pressurized neck collar to examine the relationship between aortic and carotid reflexes in human subjects. Phenylephrine was infused with and without external pressure application to the neck to cancel the effects on transmural carotid sinus pressure. This approach left the aortic baroreceptors free to respond. The carotid baroreceptors were also activated separately by neck suction. The results confirm that both reflexes participate in the control of arterial pressure in human subjects and suggest that the aortic reflex is more powerful than the carotid. The greater sensitivity applies to the control of both heart rate and adrenergic vasoconstrictor activity.

LOSS OF ARTERIAL BARORECEPTOR FUNCTION

Aksamit and colleagues described a patient with loss of carotid and aortic baroreceptor function attributable to a combination of surgery and radiation therapy.⁴⁸ Large changes in arterial pressure, induced by infusions of phenylephrine and nitroprusside, failed to affect heart rate or directly measured adrenergic vasomotor nerve activity. The patient had retained cardiopulmonary reflex activity and responded to an LBNP-induced decrease in cardiac filling with a marked increase in sympathetic nerve activity. Arterial pressure was labile, but sustained hypertension was not present. Sinoaortic de-

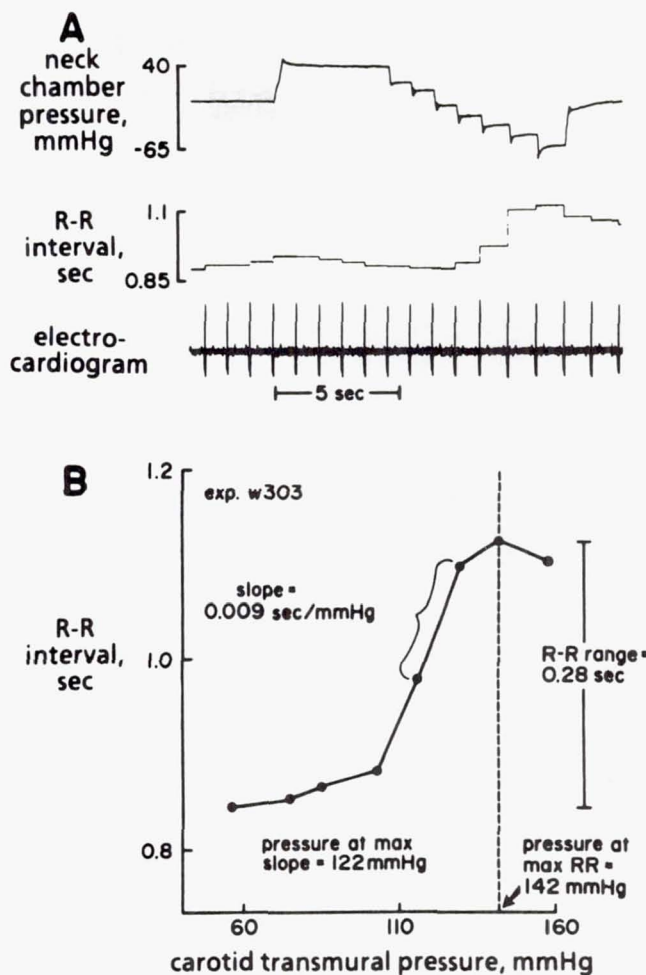


Fig. 7. Experimental record (A) and average responses of one subject to seven applications of neck pressure sequence (B). B indicates method used to analyze baroreflex relations. Carotid transmural pressure was considered to be average systolic pressure minus neck chamber pressure. Pressure at maximum slope was taken as carotid transmural pressure halfway between pressures bracketing maximum slope. (Kasting GA, Eckberg DL, Fritsch JM et al: Continuous resetting of the human carotid baroreceptor-cardiac reflex. *Am J Physiol* 252 (Regulatory Integrative Comp Physiol 21): R732-R736, 1987)

nervation in experimental animals produces a similar state. Thus, cardiopulmonary baroreceptors may contribute to the control of arterial pressure, but are by themselves unable to prevent rapid changes in arterial pressure. The patient was mildly orthostatic.

CARDIOPULMONARY RECEPTORS

The principal components of the cardiopulmonary receptor system are the left atrial and left ventricular receptors. Both sets respond to deformation. The atrial receptor population directly monitors atrial filling and indirectly monitors ventricular filling. The ventricular receptors discharge primarily during systole, but are also influenced by diastolic events. Changes in ventricular wall stress, which is maximal during isovolumic systole, may be the primary stimulus.

There are numerous and complex interactions between the mechanisms maintaining arterial pressure and body fluid homeostasis. Arterial pressure levels directly affect tissue filtration rates and renal excretion of sodium and water. The arterial and cardiopulmonary baroreflexes also control renal sympathetic activity (α -adrenergic vasoconstriction, β_1 -mediated activation of the renin-angiotensin system). Vasopressin (antidiuretic hormone) is released from the neurohypophysis in response to increases in plasma osmolarity as detected by receptors in the hypothalamus. Vasopressin is also released when the atrial mechanoreceptors are unloaded by decreasing filling pressures, usually as a consequence of decreased central blood volume. Unloading of ventricular and arterial baroreceptors by decreases in transmural pressures also releases vasopressin. The relative importance of these receptor sites is not known in detail, but the atrial release mechanism may be less active in primates than in other species. Vasopressin may be physiologically important as a vasoactive substance, inducing vasoconstriction in skeletal muscle and the splanchnic area and vasodilatation in the coronary and cerebral circulations by a combination of endothelium-dependent (cyclo-oxygenase-mediated, indomethacin-inhibited) and direct relaxation of smooth muscle.

Release of atrial natriuretic peptide (ANP) is caused by an increase in atrial transmural pressures. In addition to inducing natriuresis, ANP has multiple effects including vasodilatation and venodilatation, inhibition of renin and vasopressin release, and perhaps also a direct effect on capillary permeability.^{17,38,60}

LBNP at nonhypotensive levels has been used as a means of unloading the low-pressure cardiopulmonary receptors without affecting the arterial sensors. LBNP in the range -5 to -10 or -15 mm Hg produces significant vasoconstriction, but there is no change in arterial systolic or diastolic pressures. Pulse pressure and aortic pulse contour also remain unchanged at moderate LBNP levels. These findings, combined with the absence of any heart rate change (see Fig. 3 and Rowell¹⁷), provide evidence for preferential involvement of the low-pressure receptor pathway and suggest that the principal response is vasoconstriction. However, cardiac filling pressures and stroke volume decrease. This is likely to cause a decrease in aortic and arterial pulse volume with a significant secondary change in carotid sinus and aortic wall stress. Some degree of activation of arterial baroreflexes cannot be ruled out, and the ventricular receptors may also respond.

LOSS OF CARDIOPULMONARY RECEPTOR FUNCTION

Current surgical technique in cardiac transplantation preserves the dorsal portion of the atria, including the neural pathways to and from the left atrial receptors. The efferent pathways to the right atrium and the sinus node are also intact, but the node is electrically isolated from the transplanted heart. The ventricular barorecep-

tors are, of course, lost. Mohanty and associates reported marked attenuation of the normal reflex-induced increases in forearm vascular resistance and plasma norepinephrine levels during LBNP after cardiac transplantation.⁶¹ The impaired responses were not caused by treatment with immunosuppressive agents. Renal transplant patients on similar regimens had enhanced vasoconstrictor responses. Furthermore, the vasomotor and norepinephrine responses to a cold pressor test were intact in the cardiac transplant patients. The combined data suggested to the authors that the impaired vasoconstrictor responses were caused by ventricular denervation. However, the patients in this series tended to be hypertensive; post-transplant patients tend to be hypertensive (as a side effect of cyclosporin treatment) and their mean forearm vascular resistance at rest was higher than in control subjects during LBNP at -40 mm Hg. Mean arterial pressure during LBNP was equally well maintained in patients and controls.

Victor and colleagues studied 12 patients after cardiac transplantation and six normal controls.⁵⁰ Left ventricular dimensions during LBNP at -14 mm Hg decreased to the same extent in both groups. There was no change in mean arterial pressure or heart rate in the control group. Muscle sympathetic nerve activity (MSNA) during LBNP, measured directly with the microelectrode technique, was twice as high as at rest. Compared with normal controls, the transplant patients had higher MSNA at rest, but an identical relative change during LBNP. Sinus rate in the atrial remnant increased by 6 beats per minute in the patients, and mean arterial pressure fell by 3 mm Hg. The increases in MSNA and sinus node rate were abolished when mean arterial pressure was kept constant during LBNP by infusion of phenylephrine. These data indicate that arterial baroreflexes can compensate for loss of the ventricular receptor function.

INTERACTIONS BETWEEN ARTERIAL AND CARDIOPULMONARY BAROREFLEXES

Vasovagal or vasodepressor syncope and orthostatic syncope in subjects with intact autonomic nervous system have many common features.^{1,5,6,39} There is an initial phase with moderate tachycardia and vasoconstriction, followed by a marked fall in heart rate and arterial pressure. There is little or no increase in plasma norepinephrine in response to the hypotension (Fig. 8). The cutaneous circulation is usually vasoconstricted, but there is a large decrease in systemic resistance caused by vasodilatation in skeletal muscle.⁷ Paradoxical vasodilatation and bradycardia are also common features of hemorrhagic shock.^{39,62} Data obtained by direct nerve recording techniques have documented a strong inhibition of impulse traffic in the α -adrenergic vasoconstrictor fibers supplying skeletal muscle during presyncope and syncope.^{45,46}

The most likely cause of this sequence of events is conflicting inputs from arterial and cardiopulmonary

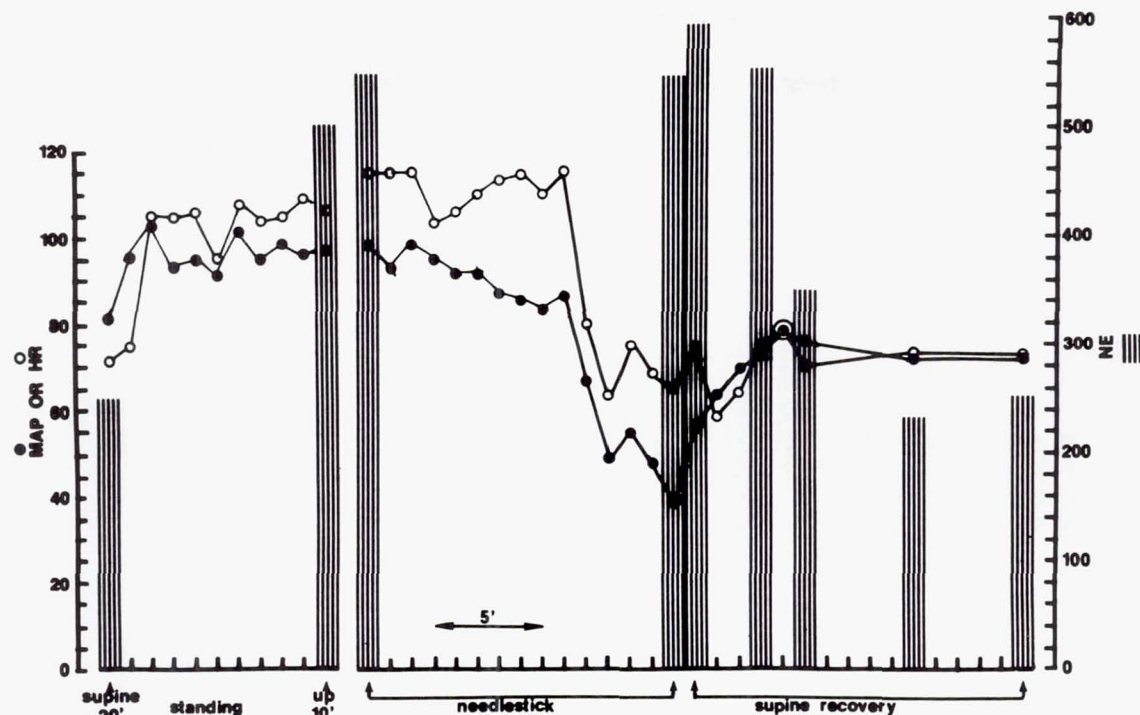


Fig. 8. Mean arterial pressure (MAP), heart rate (HR), and plasma norepinephrine (NE) concentrations during syncope evoked by the emotional response to insertion of an intravenous needle in a 17-year-old female patient who suffered from recurrent syncopal episodes. Syncope was associated with severe hypotension and bradycardia. There was no norepinephrine response to hypotension during syncope, although the norepinephrine response to standing was intact. (Goldstein DS, Spanarkel M, Pitterman A et al; Circulatory control mechanisms in vasodepressor syncope. *Am Heart J* 104:1071-1075, 1982)

baroreflexes. The left ventricular receptors are normally activated by increased intracavitary pressure and/or volume with increased wall stress. A progressive reduction in ventricular volume probably occurs during the presyncopal stage. Echocardiographic studies have demonstrated gradually decreasing left ventricular volumes with increasing degrees of peripheral venous pooling.¹⁹ The left ventricular endocardial receptors will eventually be activated by direct compression. The salient stimulus is deformation, but the sensing system cannot differentiate between compression, which is associated with low volume and pressure, and distension, which is caused by high ventricular pressure and volume. The normal adjustments to reduced cardiac output and arterial pressure are negated, and bradycardia and vasodilatation are produced. An unstable autonomic state sometimes occurs during the presyncopal phase with large oscillations in heart rate and arterial pressure (Fig. 9).⁶³ This may reflect variations in the balance between opposing drives from ventricular and arterial receptors (*i.e.*, deformation of the ventricular receptors in an empty heart falsely signaling high left ventricular pressures at a time when the carotid and aortic receptors sense a low arterial pressure¹⁶) or represent an exaggeration of the intrinsic 0.1 Hz cyclical variations in adrenergic vasomotor activity.⁶⁴

There is strong collateral support for an important role for the ventricular baroreceptors. β -Adrenergic blockade increases left ventricular end-diastolic and endsystolic volumes and improves orthostatic tolerance after bed rest.⁶⁵ Activation of ventricular deformation receptors by high ventricular transmural pressure or direct contact is likely to be the principal cause of syncope in aortic stenosis and in idiopathic hypertrophic subaortic stenosis.³⁹ Bradycardia and arterial hypotension are also common features during the early stages of an acute inferior or inferoposterior myocardial infarction.³⁹ The activity of ventricular mechanoreceptors is likely to be enhanced by increased deformation of the ischemic segment of the ventricular wall. Reflex inhibition of renal sympathetic activity may, at least theoretically, limit the ability to conserve intravascular volume and to enhance vasoconstrictor responses (no renal vasoconstriction and no activation of the renin-angiotensin system). The hemodynamic effects at rest are usually transient, but relative bradycardia and hypotension are often present during the standard submaximal exercise test at discharge. The attenuated exercise responses are usually normalized within a few weeks when the healing process is completed (unpublished observations), and there is likely to be less deformation in or at the edge of the infarcted area.

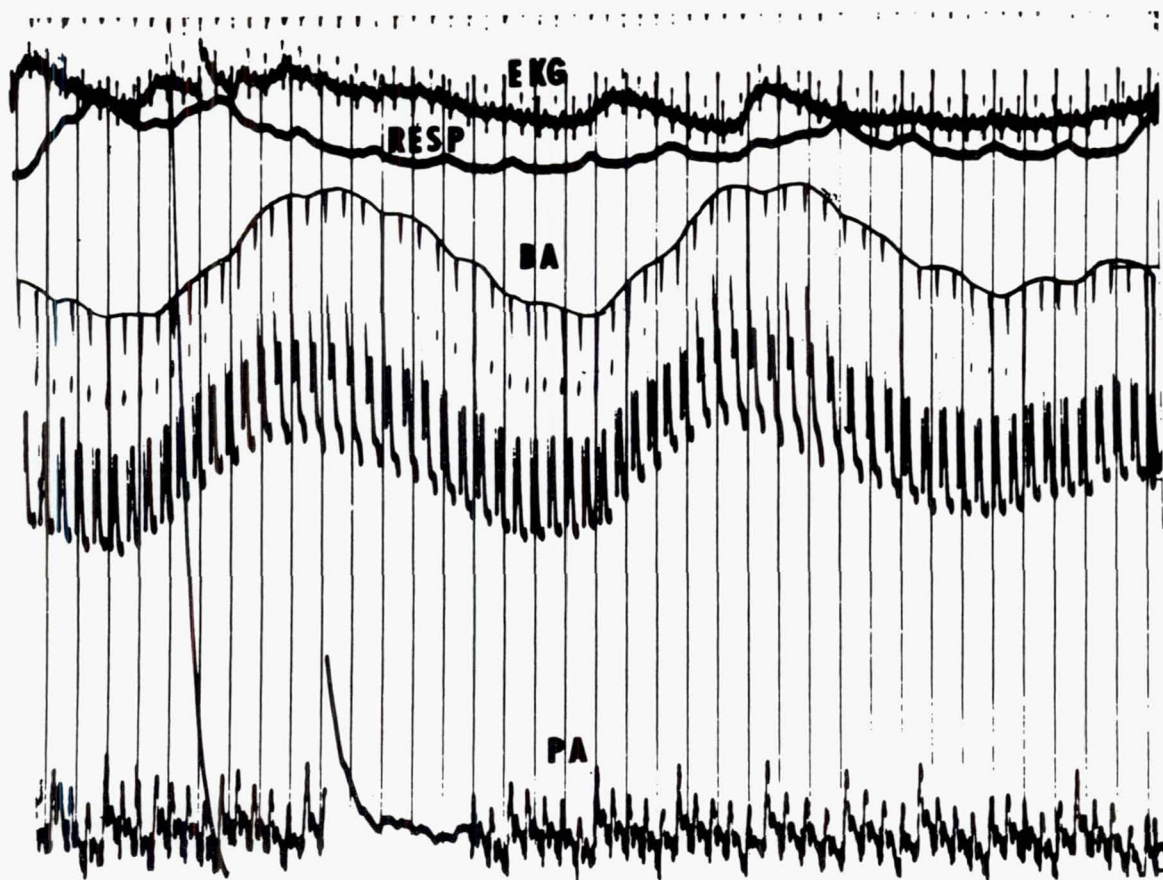


Fig. 9. Vasomotor waves are present in the brachial artery pressure tracing (BA) but are not seen in the pulmonary artery pressure tracing (PA). Waves are unrelated to respiration (RESP) and are now believed to represent variations in α -adrenergic activity. The periodicity usually approximates 0.1 Hz. These waves were recorded during 70° head-up tilt after a 14-day bed rest period in a subject with reduced orthostatic tolerance. (Hyatt KH: Hemodynamic and body fluid alterations induced by bed rest. In Murray RM, McCally M (eds): *Hypogravic and Hypodynamic Environments*, pp 187-209. Washington, DC, National Aeronautics and Space Administration, 1971)

CLINICAL ASPECTS OF ORTHOSTATIC HYPOTENSION

EFFECT OF AGING

Orthostatic hypotension from all causes becomes more prevalent with increasing age.⁶⁶ Caird and colleagues studied a large group of ambulatory men and women aged 65 and older.⁶⁷ Decreases in systolic blood pressure to 20+ mm Hg below supine resting levels after 1 minute of standing occurred in 24% and decreases of 30+ mm Hg occurred in 9% of the study population. A majority of the subjects had (1) two or more conditions likely to be associated either with hypovolemia or maldistribution of the blood volume (*e.g.*, anemia, chronic infection, or varicose veins) or with impaired cardiovascular control mechanisms (*e.g.*, attributable to treatment with pharmacologic agents having a known potential to cause orthostatic hypotension, such as levodopa, phenothiazines, tricyclic antidepressants, and vasodilators) or (2) presence evidence of structural neurologic lesions.

Cardiovascular control mechanisms tend to have reduced efficiency even in generally healthy older persons. Changes in arterial pressure produce a smaller heart rate response than in younger subjects, suggesting a blunting of the arterial baroreflex.^{68,69} Aging also attenuates responses mediated by β_1 -adrenoceptors. There is no conclusive information on the effect of age on α -receptor characteristics and responses to exogenous α -adrenergic stimulation or on humoral mechanisms modulating effector responses (*i.e.*, locally released or circulating prostaglandin, kinins, angiotensin, etc.).⁷⁰

EFFECT OF PHYSICAL FITNESS AND EXERCISE

A possible inverse relationship between physical fitness and orthostatic tolerance has been identified by Klein and co-workers⁷¹ and Stegemann and associates⁷² and has been studied extensively. One important reason for this interest is simply that physical fitness is usually perceived as a state with increased ability to withstand

TABLE 2. Postexercise Hemodynamic Data in Six Normal Subjects

Variable	Preexercise Control Value	Postexercise Measurements			
		5 Minutes	25 Minutes	50 Minutes	110 Minutes
Heart rate (beats per minute)	60	105*	89*	79*	74*
Mean arterial pressure (mm Hg)	94	90*	88*	87*	93
Central venous pressure (mm Hg)	6	4*	3*	4*	4*
Bicarbonate (mmol/liter)	24	15*	20*	23	24
Plasma volume (%)	100	84*	89*	98	100

* $p < 0.05$, compared with control values. All measurements were taken with the subject in the supine position. (Data from Bjurstedt H, Rosenhamer G, Balldin U, et al: Orthostatic reactions during recovery from exhaustive exercise of short duration. *Acta Physiol Scand* 119:25-31, 1983)

stress, particularly stress in the form of environmental extremes.⁷¹ Decreased orthostatic tolerance is then paradoxical, particularly in a condition associated with expanded blood volume, large heart size, and large functional reserves that could be used to compensate for decreased filling by increased heart rate and peripheral resistance. The paradox has been heightened by the fact that physical deconditioning by bed rest inevitably produces orthostatic intolerance.

Most, but not all,⁷³ investigators have found fitness-related differences in orthostatic tolerance, but the mechanisms are poorly understood. Early data indicated increased degree of peripheral venous pooling in fit subjects,⁷⁴ but later work has provided only limited support for increased venous compliance.⁷⁵ Several cross-sectional and longitudinal studies have examined various aspects of baroreceptor function. Fit persons have been shown to have attenuated heart rate^{71,74,76-78} and vasoconstrictor responses to orthostatic stress.⁷⁷⁻⁷⁹ Corresponding findings have been made in experimental animals.⁸⁰

Significant group differences in orthostatic tolerance also have been reported in the absence of any major difference in baroreflex function.⁸¹ It is possible that the decreased orthostatic tolerance to a significant extent is a consequence of cardiac mechanics rather than neurohumoral regulatory adaptations. Physical training alters the effective ventricular pressure-volume relationships. Fit subjects are able to respond to increased ventricular filling during exercise with a larger increase in stroke volume than sedentary persons.³⁵ This implies that the ventricle operates on the steep portion of its pressure-volume curve (see Fig. 5) and that the functionally favorable effect of increased filling is balanced by a correspondingly large decrease in end-diastolic volume and stroke volume when filling pressure decreases in the upright position. A major role of mechanical diastolic mechanisms also may help explain why the very fit and the unfit tend to have orthostatic intolerance whereas there is little or no relationship between tolerance and fitness in the mid range.

The relationship between fitness and orthostatic tolerance has important practical implications in aerospace medicine. Modern high-performance military aircraft are able to withstand considerably higher G-force levels (upward of 9G) than their pilots. A rapid

increase in +Gz forces (head-to-foot acceleration) can produce a sudden decrease in cerebral perfusion and sudden loss of consciousness with incapacitation. Full recovery may take as long as 30 seconds with catastrophic consequences.^{82,83} Straining maneuvers and isometric muscle activity during acceleration stress can substantially improve G tolerance and require a high level of general fitness, but extreme aerobic fitness may be counterproductive. Optimal exercise training regimens are yet to be defined, although a balanced approach seems preferable. The principal beneficial effect of aerobic exercise, defined as prolonged efforts involving large muscle groups in primarily dynamic exercise, is probably an increase in blood volume. This may be counterbalanced by increased peripheral venous pooling and training effects on diastolic myocardial mechanics. An activity program that promotes both aerobic fitness and the development of skeletal muscle mass and strength has a greater potential to be effective. Convertino and associates⁸⁴ have reported favorable effects of a brief bicycle-based training program that produced a modest increase in maximal oxygen uptake, whereas Pawelczyk and co-workers⁷⁵ found decreased tolerance after a running program.

Heavy exercise also has acute effects on orthostatic tolerance by producing a combination of transiently increased body temperature, metabolic acidosis, and hypovolemia with reduced central venous pressure and mean arterial pressure (Table 2).⁸⁵ This phase is followed by increased orthostatic tolerance, probably due to an expansion of the plasma volume.⁸⁶

HYPERREACTIVE HYPOVOLEMIC ORTHOSTATIC HYPOTENSION

ORTHOSTATIC INTOLERANCE CAUSED BY PROLONGED BED REST AND RELATED CONDITIONS

Prolonged bed rest is a common cause of orthostatic intolerance and decreased exercise performance.^{10,87-89} The hemodynamic syndrome is of the hypovolemic hyperreactive variety. There is generally only a modest loss of blood volume (300 to 500 ml), and the degree of hemodynamic abnormality is greater than predicted

from the magnitude of the hypovolemia. The development of cardiovascular dysfunction during bed rest has generally been attributed to the prolonged physical inactivity, but there is now strong support for the concept that a rapid response to the redistribution of body fluids is the primary mechanism.⁹⁰⁻⁹² Head-down tilt at moderate degrees was first introduced in the Soviet Union as a means of simulating the redistribution of fluids that occurs at zero gravity.⁹³ A 20- to 24-hour period of tilt at -4° to -6° produces a marked central shift of intravascular and interstitial fluid. Central venous pressure, left ventricular end-diastolic volume, and stroke volume all increase transiently, but the increased central volume promptly activates various compensatory mechanisms. There is also a significant humoral response with inhibition of vasopressin, renin, and aldosterone.⁹⁰

A negative fluid balance is established within hours during head-down tilt. Filling pressures, stroke volume, and cardiac dimensions decrease to a level below the supine baseline within 24 hours.⁹⁰⁻⁹² In fact, at that time the hemodynamic state in the supine position is similar to that normally prevailing in the upright position. When the system is challenged with an intravenous volume load, the disposition of the infused volume is similar before and after head-down tilt with an equally rapid return to preinfusion intravascular volume in both states despite the significant tilt-induced hypovolemia.⁹⁴ This implies that adaptation produces a new operating point for the mechanisms controlling intravascular and interstitial volume. These observations are consistent with Gauer's view (see Blomqvist and Stone¹⁰) that the upright position defines the normal operating point for the human cardiovascular system. Once adaptation has occurred and supine hemodynamics approach the normal upright pattern, the subject will have lost the capacity to deal with the fluid shift that occurs during the transition from supine to upright position. Orthostatic intolerance becomes manifest. The degree of cardiovascular dysfunction is similar after a 3-week bed rest period and after 20 hours at head-down tilt.⁹² A similar sequence of events is likely to occur during adaptation to the microgravity during space flight. Postflight orthostatic intolerance is to some extent present in virtually all returning astronauts. The degree of orthostatic intolerance and the loss of exercise capacity following space flight is also significantly greater than would be predicted from the total blood volume loss. It has been shown that blood volume loss during bed rest can be prevented by the administration of 9α -fluorohydrocortisone or corrected by intravenous fluid administration. Neither intervention completely restores normal hemodynamics.¹⁰ Exercise in the supine position during bed rest does not prevent the development of orthostatic intolerance, whereas a few hours per day spent in the standing or sitting position is an effective countermeasure.¹⁰ Relative short daily periods of LBNP at moderate levels of negative pressure have been shown to be effective in preventing orthostatic intolerance induced by prolonged (120 days) periods of head-down tilt⁹⁵

and have also been used routinely by Soviet cosmonauts during long space flights.⁹⁶

The exact regulatory adaptations that are responsible for the disproportionately large effect of the hypovolemia are still to be defined. On the other hand, there is little doubt that the fluid shift is the primary stimulus to the cardiovascular changes that develop during bed rest. This has clinical relevance and provides a rationale for reemphasis of the arm chair approach to the treatment of acute cardiovascular disorders as described by Levine and Lown.⁹⁷

MITRAL VALVE PROLAPSE AND RELATED CONDITIONS

Much attention has been paid to a fairly large, but poorly defined, group of patients with functionally important circulatory abnormalities in the absence of any structural neurologic or major cardiovascular lesions. Symptoms suggesting orthostatic intolerance are common. Other complaints include atypical chest pain, palpitations, fatigue, and poor exercise tolerance. In the absence of any physical or echocardiographic findings of mitral valve prolapse (MVP), these patients are often given diagnosis of dysautonomia, vasoregulatory asthenia,⁹⁸ or hyperkinetic heart syndrome⁹⁹ or are considered to have cardiovascular symptoms related to anxiety neurosis. Starr¹⁰⁰ suggested that the primary defect in neurocirculatory asthenia is a "clumsiness of the circulation," analogous to the ordinary clumsiness of muscular movements. Clumsiness in a sense of lack of precise control is a prominent feature of the mitral valve prolapse syndrome (MVPS, the combination of prolapse and symptomatic autonomic dysfunction) and related disorders. Some patients with MVPS have either markedly attenuated or grossly enhanced vagally mediated cardiovascular responses to common stimuli, such as the Valsalva maneuver or the diving reflex.^{24,101}

Many aspects of MVPS have been examined in great detail.¹⁰² A series of studies by Gaffney, Schutte, and associates have dealt with the nature of the autonomic dysfunction in MVP, including its links to the degree of valvular abnormality and its relation to similar functional abnormalities in patients without valvular defects.^{24,103-106} The combined experience of these investigators has been reviewed.¹⁰⁷

There is a tenuous relationship between the degree of anatomical abnormality and the severity of any symptoms. The characteristic click-murmur complex is only a marker that reflects an abnormal relationship between valvular and ventricular anatomy. Prolapse can be the consequence of a redundant valve or of reduced left ventricular size. At one extreme is a group of patients with a large valve and associated skeletal defects, including pectus excavatum and scoliosis. Schutte and co-workers described a distinctive habitus in women with MVP.¹⁰⁴ A discriminant function that used only height, arm span, and anteroposterior chest diameter produced correct classification of 75% to 85% of patients with MVP and controls. The combination

of prolapse and these anthropomorphic features is inherited as a dominant trait. On the opposite side of the spectrum are patients who may be symptomatic with chest pain, palpitations, fatigue, exercise intolerance, and marked orthostatic hypotension and who have prolapse with normal valvular anatomy, but a small left ventricle. Furthermore, MVP can be produced in perfectly normal asymptomatic persons by interventions that decrease the size of the left ventricle. Beattie and co-workers performed two-dimensional echocardiograms in 20 normal subjects during LBNP that induced a progressive reduction in left ventricular volume.¹⁰⁸ Almost one third of the subjects developed posterior bowing of the mitral leaflets and fulfilled classic echocardiographic criteria for MVP.

It has been suggested that many patients with prolapse have a primary hyperadrenergic state,^{102,109,110} expressed primarily as increased β -adrenergic activity that produces a hyperkinetic circulatory state. However, most of our patients with MVP have had normal levels of plasma catecholamines and normal hemodynamic state during supine rest. The heart rate response to exogenous β -adrenergic stimulation by infusion of isoproterenol is also within normal limits. Some patients show large postural increases in plasma norepinephrine levels, but these persons tend to have large postural decreases in ventricular end-diastolic volume and stroke volume. Massive sympathetic activation with tachycardia and vasoconstriction is necessary to maintain normal blood pressure and cerebral perfusion in the upright position. However, some patients have an exaggerated vasoconstriction and produce blood pressures above control values even in the presence of an abnormally low cardiac output, suggesting true α -adrenergic hyperreactivity. Maintaining a normal activity pattern and spending the day in the upright position, sitting, standing, and walking then produces a chronic hyperadrenergic state.

Hypovolemia is a common feature of the prolapse syndrome. The combination of increased α -adrenergic activity and hypovolemia in MVPS is reminiscent of the findings in patients with pheochromocytoma, in whom excessive catecholamines cause a volume-contracted state. Other studies in normotensive and hypertensive subjects have also documented a strong, general, inverse relationship between blood volume and the levels of sympathetic stimulation. Increased vascular tone in both arterial and venous systems reliably produces a rapid and marked decrease in total blood volume. The hypovolemia will become chronic if the increase in sympathetic drive persists. Mechanisms by which chronic vasoconstriction, hypovolemia, and MVP and MVPS might interact are presented in Figure 10.

The hypovolemia and MVP combine to magnify the reduction in forward stroke volume that normally occurs during orthostatic stress. A vicious cycle is established when marked vasoconstriction is required to maintain arterial blood pressure and cerebral perfusion in the upright position. Substantial mitral regurgitation is not a prerequisite for an exaggerated postural stroke

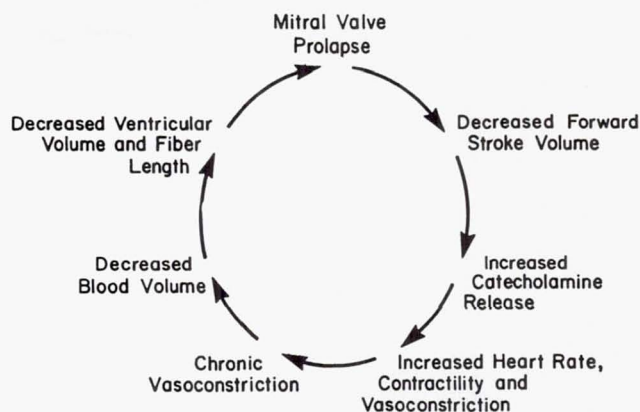


Fig. 10. A proposed set of pathophysiologic mechanisms linking mitral valve prolapse and autonomic nervous system dysfunction in a vicious cycle. A hemodynamically significant prolapse is not a requirement. (Gaffney FA, Blomqvist CG: Mitral valve prolapse and autonomic nervous system dysfunction: A pathophysiological link. In Boudoulas H, Wooley CF (eds): *Mitral Valve Prolapse and the Mitral Valve Prolapse Syndrome*, pp 427–443. Mount Kisco, NY, Futura Publishing Co, 1988)

volume reduction. The increasing volume contained by the ballooning mitral leaflets with decreasing ventricular size may produce, for any given reduction in left ventricular filling pressure, an exaggerated decrease in diastolic sarcomere length, fiber shortening, and forward stroke volume. These effects are likely to be further amplified by effects of hypovolemia on effective ventricular pressure–volume relationships (see Fig. 5). Measurements based on radionuclide ventriculography have shown marked reduction in left ventricular end-diastolic volume in patients with MVP during upright rest and exercise. This supports the concept that decreased ventricular filling and forward stroke volume in the upright position are critical features in the pathophysiology of this syndrome.^{24,107}

This relationship between MVP, reduced blood volume, and chronic vasoconstriction may well provide an explanation for the complex overlap of features in MVP and in a variety of functional and psychiatric syndromes.¹⁰⁸ Excessive vasoconstriction caused by chronic anxiety with elevated catecholamines, high resting heart rates, and diminished plasma and ventricular volumes may produce functional MVP defined as abnormal motion of a structurally normal mitral valve. Similarly, autonomic dysfunction with orthostatic intolerance in patients with myxomatous MVP could be expected to increase the frequency of symptoms, such as palpitations, easy fatigability, near-syncope, and resting tachycardia that often are interpreted as signs of psychoneurosis. Although studies specifically linking anxiety, vasoconstriction, and diminished blood volume are not available, a number of psychophysiologic studies document a strong relationship between acute and chronic stress, anxiety, and vasoconstriction. This relationship forms the rationale for the use of skin temperature as an indicator of the levels of stress and anxiety when training subjects in relaxation

TABLE 3. General Classification of Autonomic Failure

- I. Primary
 - A. Pure autonomic failure (Bradbury-Eggleston syndrome, formerly idiopathic orthostatic hypotension)
 - B. Autonomic failure with multiple system atrophy (Shy-Drager syndrome)
 - C. Autonomic failure with Parkinson's disease
- II. Secondary
 - A. General medical disorders (diabetes, amyloid, carcinoma, alcoholism)
 - B. Autoimmune diseases (acute and subacute dysautonomia, Guillain-Barré syndrome, connective tissue diseases)
 - C. Metabolic diseases (porphyria, vitamin B₁₂ deficiency, Tangier disease, Fabry's disease)
 - D. Hereditary disorders (dominant or recessive sensory neuropathies, familial dysautonomia, familial hyperbradykinesia)
 - E. Central nervous system infections (syphilis, Chagas' disease, herpes zoster, human immunodeficiency virus)
 - F. Central nervous system lesions (vascular lesions or tumors involving hypothalamus or midbrain, multiple sclerosis, Wernicke's encephalopathy, Adie's syndrome)
 - G. Neurotransmitter defects (Dopamine β -hydroxylase deficiency)
 - H. Aging
- III. Drugs
 - A. Tranquilizers (phenothiazines, barbiturates)
 - B. Antidepressants (tricyclics, monoamine oxidase inhibitors)
 - C. Vasodilators (nitrates, hydralazine, calcium antagonists)
 - D. Adrenergic blocking agents (central or peripheral action)
 - E. Angiotensin-converting enzyme inhibitors

(Modified from Bannister R, Mathias C: Management of postural hypotension. In Bannister R (ed): *Autonomic Failure: A Textbook of Clinical Disorders of the Autonomic Nervous System*, 2nd ed, pp 569-595. Oxford, Oxford University Press, 1988)

techniques and biofeedback. There is also evidence that hypovolemia can be found in patients with severe, chronic stress and anxiety directly related to serious somatic disease. The "missing blood syndrome"¹¹¹ refers to a profound hypovolemia in wounded Vietnam war casualties undergoing long-term reconstructive treatment. This "anemia" is actually a severe hypovolemia, characterized by a near-normal or even slightly elevated hematocrit. It is resistant to transfusion and iron therapy and is associated with significant hypotension during surgery. It eventually disappears spontaneously when the patient's underlying condition has improved to a point when he otherwise is ready for discharge home.

Fouad and associates¹¹² have described a previously unknown variety of hypovolemia by studying a group of 11 patients with orthostatic intolerance and a marked reduction (average -27%) in blood volume. Extensive diagnostic studies excluded pheochromocytoma and hypoaldosteronism. The hemodynamic pattern at rest supine was characterized by subnormal cardiac output and high peripheral resistance. The blood pressure tended to be labile, but catecholamine responses to head-up tilt and cardiovascular responses to the Valsalva maneuver, to the cold pressor test, and to exogenous β -adrenergic stimulation were all appropriate. The hemodynamic state at rest was temporarily normalized by blood volume expansion by intravenous human albumin. The syndrome was termed *idio-*

pathic hypovolemia in the absence of any identifiable cause of the abnormal cardiovascular state.

NORMOVOLEMIC HYPOREACTIVE ORTHOSTATIC HYPOTENSION

There is a wide spectrum of neurogenic causes of orthostatic hypotension. Bannister's¹¹³ classification (Table 3) of autonomic failure includes (1) *primary defects*, in which the disease process is well defined and involves only a limited number of structural elements, (2) *secondary defects*, in which the involvement of the autonomic nervous system is part of a more general process, and, (3) *drug-induced autonomic failure*.

Orthostatic hypotension is often the first symptom of autonomic failure. Bannister suggested that the need for precise postural adjustments of the circulation arose during a late evolutionary stage and that the mechanisms preventing orthostatic cerebral ischemia therefore are less robust than other more basic control systems. However, many patients with autonomic failure present with apparent Parkinson's disease or with bladder symptoms and impotence. Most of the different conditions listed in Table 3 are discussed in great detail in a monograph by Schatz¹¹⁴ and in Bannister's textbook.¹¹³

Schatz classified the neurogenic causes with respect to the anatomical site of the principal defect.¹¹⁴ Involvement of afferent pathways is relatively rare, but occurs in diabetes mellitus, alcoholic neuropathy, and the Holmes-Adie syndrome. Central lesions cause the autonomic failure in familial dysautonomia (Riley-Day syndrome). Multiple cerebral infarcts and Wernicke's encephalopathy may induce autonomic dysfunction with orthostatic hypotension. Mild orthostatic hypotension is also often present in idiopathic parkinsonism.

The majority of the causes of neurogenic orthostatic hypotension primarily involve the efferent pathways of the autonomic nervous system. Pure autonomic failure (formerly idiopathic orthostatic hypotension) is characterized by denervation-type hypersensitivity to direct-acting catecholamines, but decreased response to tyramine and by low peripheral catecholamine stores but increased α -adrenergic receptor density, all of which are features consistent with a postsynaptic lesion. Multiple system atrophy (Shy-Drager syndrome) is a more diffuse degenerative process. Abnormalities have been documented in several areas, including the solitary nucleus and preganglionic vagal neurons. Norepinephrine levels at rest are normal, and the peripheral sympathetic system is probably intact. Spinal cord trauma may affect the function of the intermediolateral column and produce orthostatic hypotension.

Autonomic failure is often generalized in diabetes, and orthostatic hypotension may be a relatively late manifestation. Its emergence is usually caused by sympathetic vasoconstrictor nerve damage. Diabetic neuropathy may also involve afferent pathways. Any peripheral neuropathy may damage the adrenergic

vasoconstrictor nerves. Chronic alcoholism may affect both the afferent and efferent limbs of the autonomic nervous system, but orthostatic hypotension usually occurs late.

TREATMENT OF CHRONIC ORTHOSTATIC HYPOTENSION

THERAPY FOR HYPOVOLEMIC HYPERREACTIVE ORTHOSTATIC HYPOTENSION

The following sequence of care is intended primarily for patients with hypovolemic hyperreactive orthostatic hypertension, such as occurs in patients with MVPS¹⁰⁷ and related conditions.

1. Information and reassurance. Many patients with orthostatic hypotension are anxious and should be given a liberal amount of attention with detailed explanations and reassurance.
2. Physical training and increased salt intake. A progressive physical fitness program is often helpful. Physical training causes a balanced increase in plasma volume and red blood cell mass. Adrenergic activity at rest and during submaximal exercise is reduced.³⁵ However, occasionally patients may have markedly impaired cardiac filling also during exercise.¹⁰³ They often have very low exercise capacity and derive little benefit from physical training when it is used as the initial intervention. These patients may respond favorably if exercise is reintroduced at a later stage of treatment. Swimming has been recommended as an ideal form of exercise. The external hydrostatic pressure effectively pre-

vents any activity-induced orthostatic symptoms, but upright water immersion must be avoided since it is a powerful diuretic agent and rapidly induces acute hypovolemia.¹⁰ With these precautions, swimming is appropriate as an initial step, but should progress to a balanced exercise program, designed to improve both aerobic fitness and skeletal muscle mass and strength.

Many persons have been impressed with the potential dangers of excess sodium chloride. Patients with MVPS, who have symptoms of chest pain and palpitations, may be particularly prone to self-imposed salt restriction, which certainly is not needed in the presence of hypovolemia and low blood pressure. On the other hand, increased salt and fluid intake is rarely effective unless combined with other measures.

3. Low-dose clonidine treatment. Clonidine is an α_2 -adrenergic agonist. It also has central effects that usually produce adrenergic inhibition. The onset of the action is gentle, and side-effects are mostly limited to sedation and dryness of the mouth. The α -antagonistic effects usually dominate in subjects with a grossly intact autonomic nervous system, and clonidine has the capacity to break the vicious circle of vasoconstriction, hypovolemia, and orthostatic intolerance in patients with MVPS or chronic anxiety.

Clonidine treatment for at least a month resulted in reduced postural catecholamine responses and relative vasodilatation in the upright position, but markedly improved orthostatic tolerance in a series of 8 patients (Fig. 11) studied by Gaffney and associates.¹⁰⁵ The treatment also caused a 12% expan-

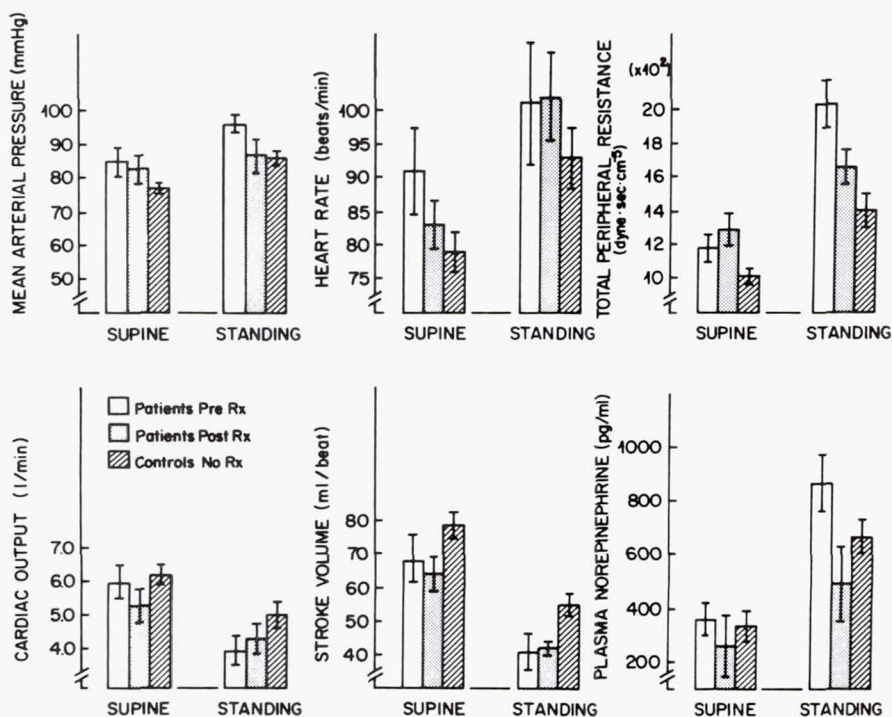


Fig. 11. Hemodynamic and neuroendocrine measurements in controls and in patients before and after long-term oral administration of clonidine (values are means \pm SE). (Gaffney FA, Lane LB, Pettinger W et al: Effects of long-term clonidine administration on the hemodynamic and neuroendocrine postural responses of patients with dysautonomia. *Chest* 83S:436S-438S, 1983)

TABLE 4. Drugs Used in the Treatment of Postural Hypotension

Site of Action	Drugs	Predominant Action
Vessels: vasoconstriction		
Adrenoceptor mediated:		
Resistance vessels	Ephedrine	Indirectly acting sympathomimetic
	Midodrine, phenylephrine, methylphenidate	Directly acting sympathomimetics
	Tyramine	Release of norepinephrine
	Clonidine	Postsynaptic α -adrenoceptor agonist
	Yohimbine	Presynaptic α_2 -adrenoceptor antagonist
Capacitance vessels	Dihydroergotamine	Direct action on α -adrenoceptors
Vessels: prevention of vasodilatation	Propranolol	Blockade of β_2 -receptors
	Indomethacin	Blockade of prostaglandins
	Metoclopramide	Blockade of dopamine
Vessels: prevention of postprandial hypotension	Caffeine	Blockade of adenosine receptors
	SMS 201-995	Blockade of vasodilator peptides
Heart: stimulation	Pindolol	Intrinsic sympathetic action
	Xamoterol	
Plasma volume expansion	Fludrocortisone	Mineralocorticoid effects
		Increased plasma volume
		Sensitization of α -receptors to norepinephrine
Kidney: reducing diuresis	Desmopressin	Action: V_2 -receptors of renal tubules

(Modified from Bannister R, Mathias C: Management of postural hypotension. In Bannister R (ed): Autonomic Failure: A Textbook of Clinical Disorders of the Autonomic Nervous System, 2nd ed, pp 569-595. Oxford, Oxford University Press, 1988)

sion of the plasma volume. Significant improvement was evident, measured both by symptoms and by quantitative analysis of the postural hemodynamic responses. Clonidine treatment progressed at 2-day intervals from 0.05 mg orally at bed time to 0.4 mg/day, or to side effects. Coghlan has successfully used a similar regimen at the University of Alabama, Birmingham (personal communication to Dr. Gaffney). The patients with idiopathic hypovolemia studied by Fouad and associates¹¹² received clonidine in low doses (0.1 to 0.2 mg/day) as an effective adjunct to plasma expansion therapy with hydrofluorocortisone (0.1 mg twice daily) and a diet high in sodium.

Thus, the central adrenergic inhibitory action of clonidine that makes it effective in essential arterial hypertension produces equally beneficial effects in hypovolemic hyperreactive orthostatic hypotension. Clonidine has also proved to be a useful agent in patients with severe idiopathic hypotension and complete loss of peripheral neural sympathetic and parasympathetic control. In these patients, the α_2 -agonist properties totally dominate and produce vasoconstriction and venoconstriction with a substantial increase in blood pressure.¹¹⁵

- Progression to the treatment usually reserved for patients with normovolemic hyporeactive orthostatic hypotension is indicated if measures 1 through 3 prove ineffective.

THERAPY FOR NORMOVOLIC HYPOREACTIVE ORTHOSTATIC HYPOTENSION

The approach to therapy is generally more complex in the hyporeactive group. It is often very difficult to determine the exact nature, localization, and extent of

the underlying disease process. As a consequence, the therapy will often have an empiric component.

Bannister and Mathias¹⁵ have reviewed general principles for management and made several important points. Patients with chronic hyporeactive orthostatic hypotension tend to adjust their autoregulatory range for adequate cerebral blood flow. They are often able to maintain adequate cerebral perfusion at subnormal arterial pressures, such as at systolic levels of about 60 mm Hg compared with 80 mm Hg in most normal subjects. Therapy should therefore be guided by symptoms and signs of cerebral ischemia rather than by the blood pressure. Furthermore, consistently normal pressures in the upright position can often only be maintained at the cost of inducing hypertension in the supine position. Comprehensive approaches to treatment have been formulated by Schatz¹¹⁴ and by Bannister and Mathias.¹⁵

GENERAL CONSIDERATIONS AND RECOMMENDATIONS

Trivial stresses can produce symptomatic hypotension in patients lacking essential elements of the blood pressure control system and include straining during micturition or defecation, exposure to a warm environment, and having an ordinary meal. Carbohydrates are more likely to induce hypotension than fats or proteins, perhaps via release of insulin and gastrointestinal hormones with vasodilator properties. Alcohol is prone to cause further vasodilatation. On the other hand, caffeine has been found to minimize postprandial hypotension in a placebo-controlled study.¹¹⁶ Vasoactive drugs should be avoided. The response to vasodilators is amplified for lack of defense mechanisms and the effects of vasoconstrictors and venoconstrictors

tors may be greatly magnified by denervation hypersensitivity.

Most patients with chronic orthostatic hypotension have a definite circadian rhythm with minimal pressures during the morning hours. Head-up tilt during sleep, first proposed by Maclean and Allen,¹¹⁷ minimizes the redistribution of body fluids that otherwise occurs at night. Normally, the central fluid shift during supine bed rest increases cardiac filling and causes a diuresis with a loss of intravascular and interstitial fluid. These losses, which tend to be abnormally large in patients with autonomic failure, are contained by the use of the head-up tilt. There is often a significant improvement of the blood pressure levels during the day and nocturnal hypertension is avoided. External support, in the form of a custom-fitted counterpressure garment, is quite effective in many patients. The garment is constructed of an elastic mesh of graded firmness to match the postural hydrostatic gradients. The disadvantages of the approach become obvious in a hot climate.

PHARMACOLOGIC APPROACHES

A summary of current pharmacologic approaches is given in Table 4.¹⁵ By the nature of these diseases, most agents have been used only in very small groups of patients, and it is difficult to provide adequate evaluation of any single specific approach. The use of clonidine has been discussed in an earlier section. Dihydroergotamine is a direct-acting α -adrenergic agonist that may preferentially cause vasoconstriction. The principal disadvantage is its poor bioavailability. Indomethacin has been used to negate the vasodilator effects of prostaglandin, but may be effective primarily by increasing smooth muscle sensitivity to norepinephrine. Fluorohydrocortisone (fludrocortisone) is the most widely used of all pharmacologic agents for the treatment of orthostatic hypotension. Its multiple actions include plasma volume expansion and sensitization of vascular receptors to pressor amines, perhaps by increasing the number of adrenergic receptors. The initial dose in autonomic failure is 0.1 mg daily.

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Cardiovascular adaptation to weightlessness

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ABSTRACT

BLOMQUIST, C. GUNNAR. Cardiovascular adaptation to weightlessness. *Med. Sci. Sports Exerc.*, Vol. 15, No. 5, pp. 428-431, 1983. Exposure to actual and simulated 0 g causes a significant central or cephalad shift of intravascular and interstitial fluid that triggers a complex set of cardiovascular and systemic adaptations. These adaptations are, in turn, directly responsible for the cardiovascular dysfunction that is apparent after return to normal gravity. However, critical information on several important adaptive mechanisms is incomplete or lacking.

An attempt will be made to resolve these problems during a future dedicated Life Sciences Space Shuttle flight. A series of cardiovascular experiments will utilize direct measurements of central venous pressures, cross-sectional echocardiography, and noninvasive measurements of systemic and peripheral blood flow at rest and during stress. Autonomic control mechanisms will be studied in detail.

WEIGHTLESSNESS, BODY FLUID DISTRIBUTION, CARDIAC FUNCTION, CARDIOVASCULAR CONTROL MECHANISMS

CURRENT CONCEPTS

The effects of gravity must be taken into account whenever a hemodynamic assessment is made. All intravascular pressures have a gravity-dependent hydrostatic component. The interaction between the gravitational field, the position of the body, and the functional characteristics of the blood vessels determines the distribution of intravascular volume. In turn, this distribution determines cardiac pump function. Observations made during and after space flight have demonstrated that exposure to 0 g causes a significant central or cephalad shift of intravascular and interstitial fluid. Cardiovascular adaptation to weightlessness is manifest postflight as actual and functional hypovolemia with orthostatic intolerance and decreased exercise capacity in the upright position (5). The central fluid shift and post-intervention hypovolemia are the salient common features of actual weightlessness and the principal simulation techniques at normal gravity, i.e., horizontal bed rest, head-down tilt, and upright water immersion (5).

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These concepts are relatively noncontroversial, but there are several important cardiovascular areas in which critical information is incomplete or lacking. A major controversy concerns the ability of the cardiovascular system to deal with the relative fluid overload associated with the central fluid shift. It has been suggested (4) that the altered hydrostatic conditions and the fluid shift trigger a complex, but rapid and effective, set of cardiovascular and systemic adaptations. Cardiovascular function is essentially normal at zero gravity, but the successful adaptation is directly responsible for the cardiovascular dysfunction that is apparent after return to normal gravity.

According to an alternative view, the normal regulatory mechanisms are unable to deal with the fluid shift. Cardiac filling pressures remain elevated for the duration of the space flight and there is a sustained hyperkinetic circulatory state. This condition has the potential of producing myocardial dysfunction.

Resolution of this controversy is a prerequisite for design of appropriate countermeasures against postflight cardiovascular dysfunction. There is also a need to know more about the effect of weightlessness on control mechanisms. A discussion of current concepts may serve as an introduction to a review of methodological approaches to be used during future space-flight experiments.

Hyperkinetic state. The hypothesis that weightlessness induces a sustained elevation of filling pressures and cardiac output is based on inflight clinical observations documenting distended neck veins and a puffy facies, often combined with subjective sensations of fullness of the head. This condition persisted over periods of up to 84 d during the Skylab flights (24). Further support has been derived from simulation studies based on water immersion in the upright position. Immersion causes an increase in central venous pressure of 10-15 mmHg and a large increase in cardiac dimensions and stroke volume. There is little or no change in heart rate. Cardiac output remains significantly elevated with no tendency to decrease even after several hours (1,2,9). On the other hand, head-down tilt at -5° produces only a transient (maximum of +2.5 cm H₂O, return to base line within 90 min) increase in central venous pressure, stroke volume, and left ventricular end-diastolic diameter without any change in cardiac output

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(4,18). It is possible that a sustained hyperkinetic state is a unique product of the hydrostatic conditions associated with immersion, and that clinical observations during flight reflect a systemic equilibration of venous pressure and regional differences with respect to tissue filtration characteristics and compliance (15) in the absence of any significant and persistent elevations of ventricular filling pressures.

Myocardial dysfunction. A sustained increase in ventricular volume and filling pressures, i.e., a volume overload, may cause myocardial damage and dysfunction as in mitral or aortic regurgitation. There is little direct evidence for a significant intrinsic myocardial dysfunction during or after space flight. The Skylab astronauts maintained or even improved their exercise capacity in space (14,17). Echocardiographic studies after the Skylab flights (13) and during simulation studies (4,18) have demonstrated a decrease in left ventricular dimensions without any effects on contractile state. However, Russian investigators (20) have described ultrastructural myocardial abnormalities affecting the mitochondria and sarcoplasmic reticulum as well as capillaries and venules in rats exposed to 0 g for 20 d. Furthermore, Yegerov (26) reported an increased left atrial (but decreased left ventricular) diameter in the Salyut-6 cosmonauts after return to normal gravity. The increase in left atrial size is compatible with a prolonged period of left ventricular overload at 0 g.

Cardiovascular control mechanisms. There is strong, if mainly indirect, evidence that adaptation to weightlessness and other hypogravic conditions alter cardiovascular control mechanisms. The blood volume loss after space flight, prolonged bed rest, immersion, and head-down tilt is generally modest, 250–500 ml. The degree of cardiovascular dysfunction is disproportionately severe. Volume-for-volume fluid replacement fails to correct completely the orthostatic intolerance (4,5).

The mechanisms responsible for orthostatic intolerance may include changes in venous compliance and in neurogenic or hormonal control mechanisms. The evidence for venous compliance changes is equivocal. The degree of pooling during lower-body negative pressure (LBNP) and occlusion plethysmography was increased during the Skylab flights, but was normal immediately after return to normal gravity (24). Simulation studies have demonstrated transient early increases, but normal compliance post-intervention (4,9).

Data on autonomic function after prolonged bed rest and space flights are inconclusive. The post-adaptive heart rate response to orthostatic stress is increased. This may be viewed as an appropriate compensation for a decrease in stroke volume. The normal vasoconstrictor responses also appear to be intact (4). Chobanian et al. (7) found no bed rest-induced changes in the pressor responses to the infusions of norepinephrine and angiotensin. Plasma catecholamines were reduced during bed rest, but the response to tilt was unchanged. The apparent turnover

rate of norepinephrine was also normal. However, Schmid et al. (22) demonstrated decreased vaso- and venoconstrictor responses to intra-arterial tyramine after a 12-d bed-rest period. There was no change in the responses to norepinephrine. These findings are consistent with normal receptor function, but impaired release of endogenous norepinephrine, perhaps due to a decreased rate of synthesis. Stone and co-workers (3,8) have recently documented reduced baroreceptor sensitivity and altered responses to vasoactive drugs after long-term horizontal immobilization in rhesus monkeys.

The combined data suggest that the orthostatic intolerance following bed rest and related conditions is a multifactorial disorder. The effects of moderate absolute hypovolemia appear to be amplified by changes in effective venous compliance and perhaps also by subtle autonomic dysfunction. Relative physical inactivity may be a contributory factor. However, the failure of even vigorous inflight exercise to prevent postflight orthostatic intolerance, and the ability to mimic important features of the postflight cardiovascular state by a short (24-h) period of head-down tilt (4), are all findings that support the crucial role of the redistribution of body fluids during the initial 1 or 2 d of space flight.

METHODOLOGY FOR CARDIOVASCULAR STUDIES AT ZERO GRAVITY

It is evident that a combination of approaches will be necessary to provide a better understanding of cardiovascular adaptive changes. The Space Shuttle system, including a dedicated laboratory module for life sciences experiments in man and experimental animals, has greatly expanded the capability to perform physiological studies in space. Nevertheless, the range of techniques that can be utilized and the number of human subjects that are available for studies will remain limited for several years to come. Studies of the same subjects at 0 and 1 g are, therefore, crucial to define appropriate simulation methods.

Progress in the cardiovascular area requires safe, accurate, and convenient methods for measurement of intracardiac and intravascular pressures, cardiac output and regional flow, cardiac dimensions and performance, and a combination of appropriate interventions and measurements for the evaluation of regulatory mechanisms. In addition, data on the spacecraft environment, diet and activity, body composition, fluid and electrolyte metabolism, blood volume, hormonal regulation, and skeletal muscle function are essential to put the cardiovascular findings into context. Such comprehensive data will be obtained by a team of investigators during the Space Lab 4 (SL-4) flight, which is scheduled for November, 1985.

Intravascular and intracardiac pressures. Operational considerations, and the need to obtain multiple observations over extended periods of time, make it necessary to

rely as much as possible on noninvasive methods. However, there are still important problems that can be resolved only by invasive procedures.

Accurate estimates of filling pressures are essential to an understanding of the cardiovascular adaptation. The most significant pressure changes are likely to occur at an early stage. It is possible that peripheral venous pressures at zero gravity accurately reflect central venous pressure (CVP) (15), but it is doubtful that peripheral measurements will provide a sufficiently-high degree of accuracy to evaluate longitudinally preflight and inflight changes. Blood volume shifts as large as 500–1000 ml may cause an increase in CVP of only 2–3 mmHg, which nevertheless has highly-significant effects on right-ventricular dimensions and performance. A central venous catheter with a very well-defined tip location is a prerequisite for an adequate evaluation. Clinical experience indicates that the risks associated with placement of a central venous catheter and right-heart catheterization is very low in healthy subjects (6). Observations may be extended over a 24-h period. Such measurements are included in the plans for SL-4 flight. We are currently developing a self-contained recording system to be worn by two payload specialists.

Simultaneous measurements of right- and left-ventricular pressures, combined with dynamic volume measurements, are desirable, but not justifiable or feasible at this time.

Cardiac output and regional flow. The most common current procedures for noninvasive measurement of cardiac output are based on one of two basically different rebreathing approaches: the CO₂ method (11) and the foreign gas method (25). The CO₂-rebreathing method (11) has many attractive features. The use of a single gas from an endogenous source represents a significant advantage in the closed environment of the Space Shuttle. The CO₂ method (11) will be used during the SL-4 experiment. Farhi and his associates (personal communication) are developing a computer-based system for measurement of cardiac output under a variety of experimental conditions, including maximal and submaximal exercise. Invasive measurements, e.g., by the thermodilution technique, are impractical during studies that require serial measurements performed over several days.

Doppler techniques may be applied to generate measurements of regional flow, at least in superficial arteries such as the carotid. More research is needed before quantitative results can be obtained. The standard plethysmographic technique for measurement of limb blood flow is currently being updated (Bhagat and Johnson, personal communication) using ultrasound to estimate changes in segmental limb volume. This technique will be used for measurement of leg volume changes during lower-body negative pressure. A standard plethysmographic technique, based on an air-filled system, will be used for inflight measurements of limb flow.

Cardiac dimensions and performance. The introduction of quantitative noninvasive cardiac imaging tech-

niques, i.e., echocardiography and scintigraphy, has had a major impact on clinical cardiology. Both techniques are likely to contribute significantly to space medicine and physiology. Current echocardiographic technology is directly applicable to inflight studies. Present scintigraphic equipment, e.g., gamma cameras, are too heavy and bulky to be ready for immediate application in space. However, recent progress in the development of lightweight imaging devices (based on multi-wire proportional counter chambers, adapted from the technology of cosmic ray physics [Lacy and Johnson, personal communication]) indicate that suitable scintigraphic devices will become available eventually.

M-mode echocardiography has been used for pre- and postflight studies (13,20) and during simulation studies (4,18). This method has serious limitations. Single-diameter measurements provide accurate estimates of wall thickness, but may generate misleading information on overall chamber dimensions, particularly if there are significant changes in chamber configuration. Cross-sectional or two-dimensional echocardiography represents a significant advantage, and is included in the 1985 Space Lab plans. Changes in chamber configuration may be recognized and a wider range of geometric models can be applied to the analysis of ventricular volumes. However, the accuracy of left-ventricular volume measurements is still less than satisfactory (12,21), and there is no valid model for quantitative right-ventricular volumes. Development work is in progress and a promising approach to three-dimensional reconstruction of left-ventricular volumes has been identified (19).

A major problem in cross-sectional echocardiography is poor definition of interfaces between blood and tissues. Advanced-image processing techniques are likely to become available. Use of an improved contrast agent, e.g., intravenous hydrogen peroxide (Gaffney, personal communication), may also prove helpful.

Regulatory mechanisms. The study of regulatory mechanisms is always difficult in the intact human subject. This is a neglected area in space physiology. The effects of weightlessness on local regulatory mechanisms are poorly understood, particularly in the important area of venous function. Current methods for measurement of venous compliance in man are primitive (23) and new approaches are needed.

Traditional techniques for evaluation of autonomic function employing blocking agents are cumbersome and also likely to interfere with inflight operational demands, mainly because of a lack of short-acting blocking agents, particularly vagolytic drugs. However, studies employing α - and β -adrenergic agonists and other vasoactive substances are feasible and included in the SL-4 flight experiment plans. Relatively simple noninvasive measurements, e.g., the heart rate response to isoproterenol or the changes in systemic and forearm conductance in response to norepinephrine or phenylephrine, will be used to derive quantitative stimulus-response relationships. As previously

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discussed, there is suggestive evidence from bed-rest studies in man and primates that adaptation to hypogravity causes measurable changes in adrenergic-agonist responses. Studies of the response to α -adrenergic antagonists are also desirable, but management of a hypotensive response may be difficult in space. Only pre- and postflight studies are being planned.

A majority of previous cardiovascular studies of complete afferent-efferent reflex arches have involved the carotid sinus mechanism. There is information from a simulation study in primates suggesting attenuated function (6), but no data on the effect of actual zero gravity. Eckberg (10) has studied baroreceptor function in health and disease, and has devised a sophisticated method that introduces brief changes in transmural carotid sinus pressure by means of a neck collar and local suction. This technique is well-suited to use in space and will be applied as an

SL-4 flight experiment. Recent advances in the study of cardiovascular receptors (16) may also prove helpful. Specific methods for evaluation of human low-pressure baroreceptor function are greatly needed, but are currently unavailable. Other more complex areas, e.g., the interactions between natriuresis, aldosterone-renin levels, and vascular reactivity also need further exploration. A comprehensive experiment in squirrel monkeys is to be performed during the SL-4 flight by Moore-Ede and co-workers (personal communication).

In summary, dedicated Life Sciences flight, SL-4, scheduled for late 1985, includes an extensive set of cardiovascular experiments which are likely to contribute significant new information on several important aspects of the cardiovascular adaptation to zero gravity, particularly on cardiac function and dimensions and on regulatory mechanisms.

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Cardiovascular adjustments to gravitational stress

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THE EFFECTS OF GRAVITY on the cardiovascular system must be taken into account whenever a hemodynamic assessment is made. All intravascular pressures have a gravity-dependent hydrostatic component. The interaction between the gravitational field, the position of the body, and the functional characteristics of the blood vessels determines the distribution of intravascular volume. In turn this distribution largely determines cardiac pump function.

Multiple control mechanisms are activated to preserve optimal tissue perfusion when the magnitude of the gravitational field or its direction relative to the body changes. Humans are particularly sensitive to such changes because of the combination of their normally erect posture and the large body mass and blood volume below the level of the heart. Current aerospace technology also exposes human subjects to extreme variations in the gravitational forces that range from zero during space travel to as much as nine-times normal during operation of high-performance military aircraft. This chapter therefore emphasizes human physiology.

HYDROSTATIC PRESSURE

Models

Hydrostatic pressure is a force acting in a fluid system as a result of a gravitational field. The hydrostatic pressure is a static pressure in both open and closed fluid systems and is added to other forces generated in the system. In the cardiovascular system

the action of the heart generates a dynamic pressure. The elastic properties and the pressure-volume characteristics of the peripheral vascular compartment modify both the dynamic and the static pressure components.

The various pressure components and their interactions are considered in a series of models of increasing complexity, starting with a simple, rigid, open cylinder. Such a cylinder of height h , filled with fluid of density ρ , has forces acting in various directions unless the fluid is completely at rest. At rest the major force acting on the fluid is a static pressure force generated by the gravitational field and directed perpendicularly to the earth's surface. The difference between the pressure at h and that at the surface of the fluid is directly proportional to h . This result can be derived by considering the balance of forces acting on some horizontal plane in the fluid (55). This force at rest is strictly vertical, implying that the horizontal components of the pressure force balance. The vertical pressure is therefore independent of the horizontal position in the cylinder. The weight of an element in the fluid at h must be balanced by an upward pressure force at the bottom of the plane. This force is greater than the downward pressure force at the top. If A is the cross-sectional area of the horizontal plane and if P_1 and P_2 are the pressures acting on the bottom and top, respectively (Fig. 1), then the net upward force on the horizontal plane is $(P_1 - P_2)A$. This pressure force equals the downward-acting weight of the fluid above the horizontal plane. The downward-acting force of the fluid equals the product of ρ , the volume (Ah'), and the force of gravity (g). Thus

$$(P_1 - P_2)A = \rho g Ah' \quad (1)$$

$$P_1 - P_2 = \rho gh'$$

If P_2 is atmospheric (P_A), the surface of the tank is at $h = 0$, and in the fluid h is negative, the pressure anywhere in the tank (P) is

$$P = P_A - \rho gh \quad (2)$$

As the depth below the surface of the tank increases, the pressure force increases. The pressure in Equation 2 is the hydrostatic pressure for a fluid at rest in a rigid tank (55).

When the same cylinder is closed rigidly at both ends and filled with fluid until all air is excluded, the pressure at the top of the cylinder is negative and approximately one-half of the pressure in the cylinder shown in Figure 1A. The pressure at the bottom is equally large but of the opposite sign (Fig. 1B) and $P = 0$ at $0.5 h$. The elastic properties of the circulatory system are disregarded by this model. Replacing both rigid ends of the closed cylinder with elastic membranes of identical properties results in a set of circumstances similar to those in the rigid, closed model (Fig. 1C). When one end of the cylinder is closed with an elastic material more rigid than that at the opposite end, however, the point in the fluid at which the

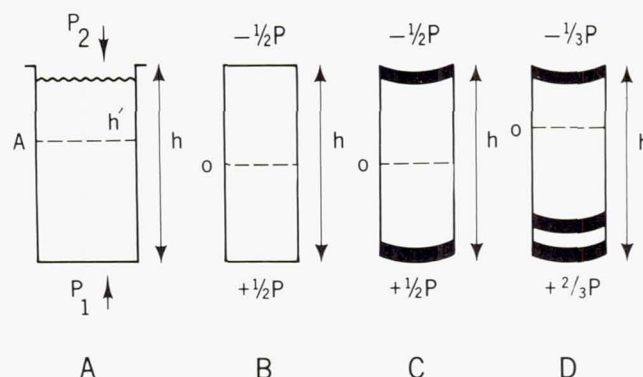


FIG. 1. Hydrostatic pressures (P) in simple fluid-filled systems of height h . Relationship of hydrostatic pressure and zero in cylinder open to atmosphere (A) and cylinders closed at both ends with rigid membranes (B), with membranes of equal elasticity at both ends (C), and with membrane more elastic at upper end (D).

hydrostatic pressure is zero moves toward the less rigid end. The magnitude of the shift in the zero point depends on the elastic properties of the ends of the cylinder (62). Including a pump in the system (e.g., heart in circulatory tree) does not change the location of the zero hydrostatic pressure point in the system but adds an additional pressure component (dynamic pressure). Thus the pressure at the top of the cylinder in Figure 1D equals the sum of $-1/3 P$ and the pressure generated by the pump.

When the cylinder is closed at both ends (Fig. 1C, D), the hydrostatic pressure at the bottom differs from that expected in an open-ended cylinder. The pressure difference between the top and the bottom corresponds to the full effective hydrostatic pressure of the open system given in Figure 1A, but part of the column in the closed system in Figure 1C is suspended from the top of the cylinder and exerts a negative pressure there. Only a portion of the total pressure difference between top and bottom is positive at the bottom of the cylinder.

In summary, three components contribute to a pressure measured anywhere within a closed fluid system, e.g., the cardiovascular system. The first component is the hydrostatic pressure, which in a stable body position is static. The complex second component reflects overall and regional elastic properties of the blood vessels, particularly the pressure-volume relationships of the veins. In general it is a static pressure but changes when the characteristics of the system are altered. The third component is the dynamic pressure that the heart generates.

Hydrostatic Indifference Point

One would assume from these considerations that during postural changes intravascular pressures in dependent regions would rise, intravascular pressures in superior regions would fall, and pressure at some intermediate point would remain constant. Pressure measurements have been made in dead and in anesthetized animals during changes from the horizontal

to the head-up and head-down positions (61, 83, 135, 225, 308). Clark et al. (61) measured intravenous pressure at several sites in dead animals. When the animals were tilted from the horizontal position either head up or head down, the equilibrium pressure equaled the sum of the hydrostatic pressure generated by the new position and the static pressure measured in the horizontal position. When the catheter tip was ~6 cm below the heart, the hydrostatic pressure was independent of body position. This point has been referred to as the hydrostatic indifference point (HIP) for the measurement of venous pressure. Anesthetized dogs produced similar results, but the HIP was 12 cm below the heart. This implies that the autonomic nervous system and changes in venous tone can influence the anatomical location of the HIP. In either case head-up tilt decreased and head-down tilt increased intracardiac pressures. However, changing the position of the HIP changes the magnitude of the pressure change at any intravascular site as a result of postural change. Guyton and Greganti (135) repeated the studies of Clark et al. (61). They placed a catheter in the right atrium and then changed the position of the tip until changes in body position produced no changes in pressure. The apparatus they used allowed them to define the position of the HIP relative to the three major axes of the body. The HIP was located within the right ventricle (Fig. 2) immediately below the level of the tricuspid valve. Interventions like reduction of blood volume, epinephrine infusion, and destruction of the spinal cord did not alter the anatomic location of the HIP for venous pressures but affected the position of the HIP for the arterial system (308). Guyton and Greganti (135) postulated that the venous HIP was located within the heart to maintain stable ventricular filling pressure and cardiac output during changes in body position. Stroke volume, however, decreases by about 30% in humans tilted from the horizontal to the head-up position (224, 260, 345) mainly because of changes in ventricular filling pressures. Thus a species difference in the response to postural changes exists. Direct measurements suggest that the anatomical location of the HIP in humans is outside the heart. Gauer and Thron (110) defined, by measurements in the horizontal position and during

head-up tilt, a venous HIP site a few centimeters below the diaphragm. Progressive head-down tilt initially caused the expected increase in cardiac filling pressures, but later it caused a paradoxical fall, implying a shift in HIP from a position below the heart to a position above the atria.

Factors such as the compliance, volume, and size of the components of the venous system determine the anatomical position of the HIP. Any change from basal conditions that affects one or more of these factors is likely to alter the position of the HIP. The pressure-volume curves of isolated veins are nonlinear and shift in response to physiological stimuli (109, 118, 185), whereas the effective systemic vascular pressure-volume relationship (as defined by volume infusion and withdrawal in intact subjects) approaches linearity (80). The pressure-volume characteristics, either through a pure volume change or through a change in the characteristics of the vessel wall (compliance), may change to a different extent in different regions. The position of the HIP theoretically should shift away from the region with a decrease in compliance. However, Guyton and Greganti (135) found that neither hemorrhagic volume depletion nor denervation by spinal ablation affected the position of the HIP in anesthetized dogs. The results are difficult to explain unless there were offsetting compliance changes caused by a combination of alterations in volume and in neural and myogenic control.

Transmural Pressure and Tissue Filtration

The three components of the pressure measured anywhere in the vascular system may be restated as 1) the hydrostatic pressure caused by the force of gravity, 2) the static filling pressure of the resting circulation (61, 134, 140), and 3) the dynamic pressure from the relationship between blood flow and resistance (229). The properties of the tissues surrounding the blood vessels and their effects on the relationship between intravascular and transmural pressures in the various portions of the vascular system should also be considered. This particularly applies to the low-pressure portions of the vascular system. The transmural pressure (P_T) is

$$P_T = P_i - P_e \quad (3)$$

where P_i is the intravascular pressure and P_e is the extravascular pressure. In *Hydrostatic Indifference Point*, p. 1026, the change in intravascular pressure with changes in body position is discussed in reference to the HIP. The effect of the gravitational field on the pressure forces outside of the vascular compartment, however, must also be taken into account. Changes in these forces may dramatically alter the effect of changes in the intravascular hydrostatic pressure component.

The tissues surrounding most of the blood vessels in the mammal are composed of 70% water, which has a specific gravity of 1. Because the outer covering of

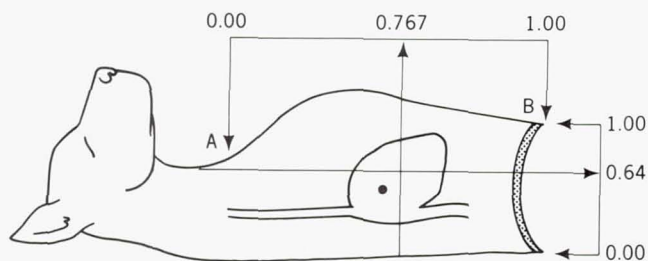


FIG. 2. Hydrostatic indifference point (HIP) in anesthetized dog is in right ventricle. A: sternal notch; B: xiphoid process. Distance between reference points is 1.00; coordinates of HIP are fractions. [Adapted from Guyton and Greganti (135).]

the body has elastic properties, it may serve as a pressure garment under many conditions. In addition many of the blood vessels pass through cavities that do not connect directly with the outside environment. Because the body is mostly water, these cavities may be expected to behave like fluid-filled systems. Changes in body position within a gravitational field would be expected to alter the pressure in these tissues in direct proportion to the vertical height of the fluid columns. From Equation 2 the expected gradient in hydrostatic pressure composed mostly of water equals 1 cmH₂O/cm of vertical height above or below the reference level.

Extravascular pressure changes with varying body position have been measured in the cerebrospinal fluid compartment (139, 216, 217) and the abdominal cavity (284). The extravascular pressure in these systems also changes with gravitational force and modifies the effective vascular pressure, i.e., the transmural pressure. In the cerebrospinal fluid system the HIP in normal subjects is located between C₇ and T₅ in the spinal

column. This location, which is similar to that of the venous HIP, tends to minimize postural changes in effective transmural vascular pressures within the central nervous system. Pressure has been measured in the pleural and pericardial cavities in conjunction with simultaneous measurements of vascular pressures in experimental animals (9-11, 68). The intravascular pressures in the right and left atrium decreased in the head-up and head-down positions more than in the supine position (Fig. 3). This would indicate that cardiac filling pressure decreased in both the head-up and head-down position because of the hydrostatic column of blood. Pressures in the pleural and pericardial cavity, however, responded similarly, and transmural cardiac pressures did not change significantly.

Changes in body position would greatly affect capillary fluid filtration if changes of similar magnitude in the extravascular pressures did not counteract the changes in intravascular hydrostatic pressure. In addition regulatory mechanisms at the microvascular level control the tissue filtration rate. The important

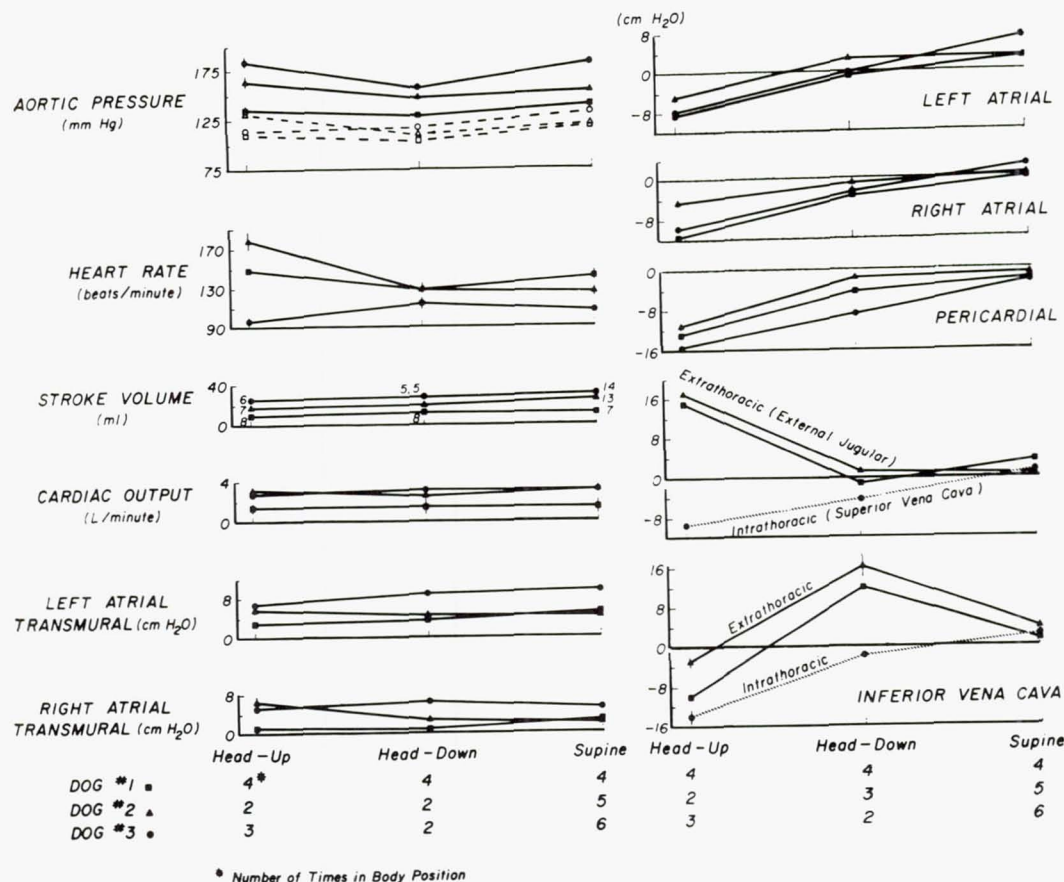


FIG. 3. Intrathoracic and venous pressure relationships and hemodynamic responses to changes in body position. Averages of right and left atrial transmural pressures, cardiac output, stroke volume, heart rate, and systemic pressures (*left*) and average abdominal vena cava, external jugular, pericardial, and right and left atrial pressures (*right*) in supine, head-down, and head-up position in 3 dogs. Adjacent to curves for stroke volume, number of cardiac-output measurements. [From Avasthey and Wood (11).]

role of venous pressure (P_v) in determining capillary pressure (P_c) can be stated as

$$P_c = P_v + R_v \cdot \frac{P_a - P_v}{R_a - R_v} \quad (4)$$

where P_a is arterial pressure, R_a is arterial resistance, and R_v is venous resistance (136, 169). Maintenance of P_c determines the bulk movement of fluid in and out of the capillary network. Recent data (165, 235) suggest that local adjustments in the relationship of pre- to postcapillary resistance are important in the microvascular response to changes in hydrostatic loading. The local response of the pre- and postcapillary vessels may include a myogenic component of the smooth muscle cells of the vessel wall (168). The principal autoregulatory adjustments occur on the arteriolar rather than the venous side. The relative contribution of metabolic and myogenic mechanisms may be different in different vascular beds (168). A change from the supine to the upright position does not normally increase extracellular fluid in dependent regions enough to produce manifest edema. Transmural pressure tends to remain constant; any change is offset by an alteration in the pre- to postcapillary resistance to maintain P_c . In conditions such as heart failure, where dependent edema can occur, the underlying cause is the increase in the dynamic value of P_v .

Evidently several mechanisms modify the hydrostatic effects of posture changes in many regions of the cardiovascular system. The best example of a simple hydrostatic system in the human is the pulmonary circulation. Regional pulmonary blood flow in normal individuals at rest depends on the pressure in the pulmonary artery generated by the heart (dynamic pressure) and the hydrostatic pressure gradient in the column of blood in the lungs (static pressure). West (352) has convincingly shown that in the upright position the blood flow to the apex of the lung is less than the blood flow to the base of the lung. In the supine position the gradient in flow is from the superior (anterior) to the dependent (posterior) regions. Coulam and Wood (68) have found a simple hydrostatic fluid pressure gradient in the pleura as body position changes. These two findings imply that transmural pressure in the lungs varies with changes in the height of the hydrostatic fluid column relative to a reference point. Blood enters the system near the center of each lung, and this level may serve as a reference point. Increasing the gravitational field should accentuate the regional differences in pressure and flow in the direction of the gravitational force vector. Conversely, removing the gravitational force should shift pressure and flow toward superior regions, such as the apex in the upright position. This hypothesis has been tested by exposing humans to 0 g in a high-performance aircraft during parabolic flight (322). Pulmonary blood flow, measured with macroaggregated human serum albumin injected before and during the parabolic flight profile, was higher in the

apical regions of the lungs (Fig. 4) during 0 g than during a seated 1- g control period.

IMMEDIATE CARDIOVASCULAR RESPONSES TO POSTURE CHANGES AND BLOOD VOLUME REDISTRIBUTION

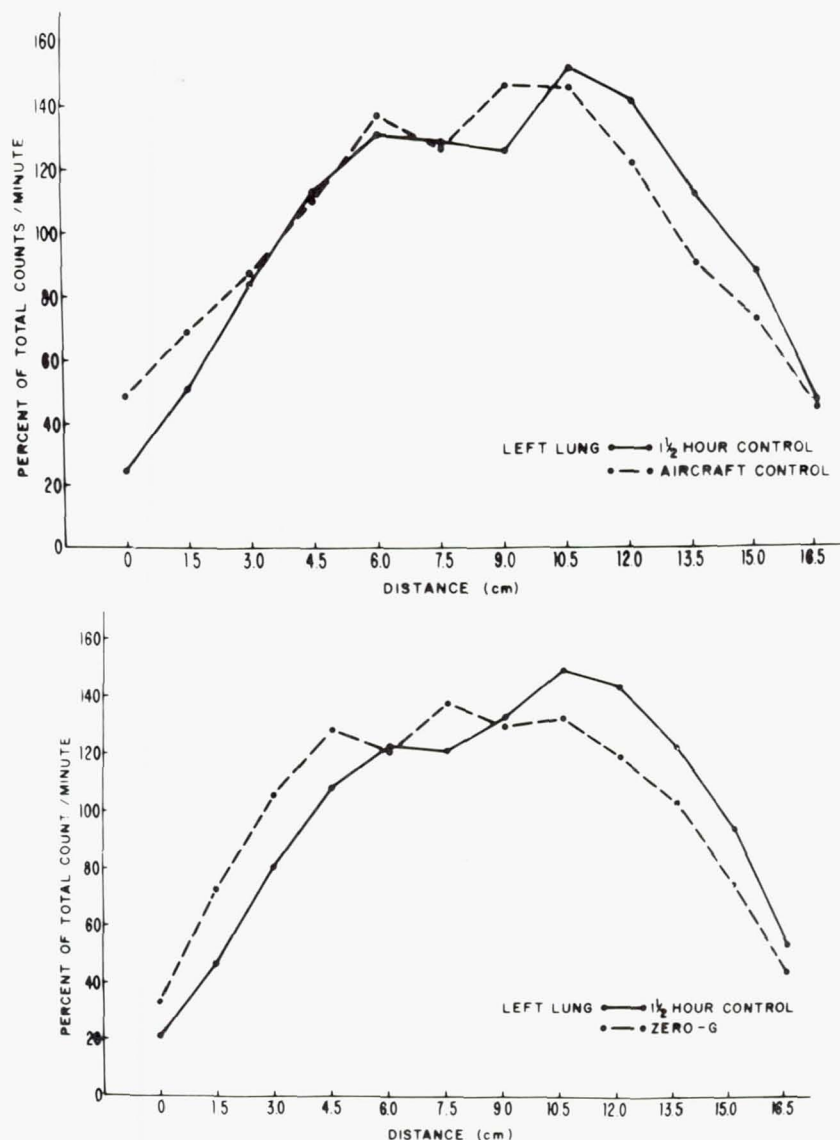
Experimental Conditions

The principal hemodynamic effect of posture change is redistribution of venous volume with marked secondary changes in ventricular filling and stroke volume. The magnitude of the volume shift is an important determinant of the physiological response. Experimental procedures that cause reproducible and quantifiable redistribution of volume are essential to the study of cardiovascular responses. Skeletal muscle activity in the legs markedly affects venous pressures and volumes (213, 214, 264). Muscle activity is difficult to control and monitor during standing. Studies of the mechanisms producing orthostatic cardiovascular changes in humans have therefore often been based on alternate methods that approximate the degree of venous pooling during standing but minimize muscle activity.

LOWER-BODY NEGATIVE PRESSURE. In aerospace medicine lower-body negative pressure (LBNP) has been used extensively as a research tool and clinical method (355). It has the inherent advantage of producing a gravity-independent redistribution of venous volume, and during space flight it can uncover changes in orthostatic tolerance (172). Furthermore during LBNP the subject remains at rest in the supine position, which facilitates physiological measurements and minimizes the likelihood of confounding activity in skeletal muscle. Measuring changes in leg volume is easy and can provide at least a semiquantitative measure of shifts in venous volume. Many different LBNP devices have been constructed, including metal cylinders and hemicylinders and wooden boxes. The bottom of the low-pressure chamber is usually fitted with a saddle to prevent movement into the device during suction. A rubber or plastic seal surrounds the subject and attaches to the opening of the LBNP chamber. The effective level of the seal is usually about the iliac crests. A commercial vacuum cleaner can produce the vacuum. A system including valves and a pressure gauge automatically or manually regulates the pressure. The degree of venous pooling is proportional to the negative pressure. In general LBNP at -50 mmHg, standing, and head-up tilt at + 70° similarly change heart rate and systolic pressure (355).

PASSIVE TILT. During passive tilt the subject rests on a table that can be rotated around an axis corresponding to a transverse body diameter, preferably at a level approximating the body's center of mass. A restraint system including a saddle prevents longitudinal changes in body position. The position of the table

FIG. 4. Regional pulmonary blood flow at 1 *g* and 0 *g* at various lung levels. Measured in volunteer by injecting radioactive tracer during 0 *g* and in seated position at 1 *g*. Note flow shifts toward apex of lungs. Similar results in 6 subjects. Zero distance, apex of lung; opposite end of scale, toward base of lung. *Top*: control measurements; *Bottom*: 0-*g* measurements.



and the subject is usually specified in terms of angular deviation from the horizontal plane, positive for head-up and negative for head-down positions. The direct hydrostatic effects are proportional to the sine of the angle between the table and the horizontal plane, i.e., +30° produces 50% and +75° as much as 95% of the effects of a full 90° change from supine to upright.

IMMERSION. This intervention produces a marked central fluid shift. Water immersion has been used extensively as a therapeutic agent (hydrotherapy) since the beginning of history. More recently immersion has been applied to investigate the renal effects of a central redistribution of blood volume (16, 17, 87-90, 105, 108) and to simulate zero gravity (121, 122).

Epstein (87) has recently reviewed the technical and physiological aspects of immersion in detail, including conditions that may modify the cardiovascular response. The temperature of the bath is critical. Ther-

moregulatory mechanisms disturb the hydrostatic effects if the bath is not thermoneutral, i.e., kept at 33°C-35°C. Water is the common medium, but silicone fluid and saline minimize fluid exchange across the skin and prevent skin lesions due to maceration. The external application of hydrostatic pressure alters respiratory mechanics. Upright immersion is equivalent to negative-pressure breathing, which by itself has significant cardiovascular and renal effects that are directionally similar to the primary vascular hydrostatic effects.

G-SUIT DEVICES. Systems with inflatable rubber bladders compressing the legs and the abdomen, called G suits, have been widely used in aviation and recently also in emergency medicine to prevent venous pooling and produce a central blood volume shift in hypovolemic shock. Recent hemodynamic studies (103) have shown, however, that the primary effect is an increase

of systemic resistance rather than an increase in ventricular filling.

LOWER-BODY POSITIVE PRESSURE. Applied with a chamber analogous to an LBNP apparatus, lower-body positive pressure (LBPP) has been used to study the renal and hormonal responses to a central fluid shift in primates (239).

Blood Volume and Distribution

Total blood volume in mammals is an essentially linear function of total body weight, but there are fairly large species differences. Physically active animals generally have larger volumes than inactive animals. Mean values in normal adult humans cluster around 75 ml/kg body wt, or 5–5.5 liters for a 70-kg man. Sources of interindividual variations include body composition, age, climate, and physical activity (306).

Available estimates on the normal distribution of blood volume in the human are based on incomplete regional measurements and extrapolation from more precise measurements in experimental animals. Approximately 70% of the total blood volume is contained in the systemic veins; the heart and lungs account for about 15%, the systemic arteries for 10%, and the capillaries for 5% (107, 124, 298, 307). Hydrostatic pressure changes and regional variations in compliance are the primary determinants of the pattern of postural redistribution of intravascular volume. There are important modifiers, however, including the compliance of perivascular tissues, hydrostatic changes in tissue pressure, and myogenic and neurogenic effects on venous tone. Also vasomotor activity causes postural redistribution of venous volume (48, 53, 254, 277).

The term *compliance* is often applied without being rigorously defined. In common usage within this subject area, it is equivalent to the change in volume for a given pressure change (dV/dP) within the physiological pressure range. Nonlinearities in the pressure-volume relationship are often disregarded. The systemic veins supply most of the overall compliance of the cardiovascular system. Effective total vascular compliance, as derived from measurements of central venous pressure during acute changes in blood volume, is of the order 2–3 ml·mmHg⁻¹·kg⁻¹ body wt in the human and dog (80, 108, 140, 189, 299). Measuring systemic vascular compliance in the intact subject is complicated by reflex-induced hemodynamic adjustments, delayed compliance (i.e., viscoelastic creep of vessel walls), and blood volume changes caused by tissue filtration (7, 358). Nevertheless the low-pressure compartment of the circulation, i.e., the systemic veins, the right heart, the pulmonary circulation, and the left atrium, which together contain 85% of the total blood volume, clearly contributes an even larger percent of total vascular compliance. The pulmonary veins account for most of the lung blood volume and the total compliance of the pulmonary circuit. The

pulmonary capillaries are relatively noncompliant (72, 86, 360). Total pulmonary vascular compliance in the rabbit is ~0.1 ml·mmHg⁻¹·kg⁻¹ body wt (86). Compliance of the systemic arteries is lower by an order of magnitude, or ~0.01 ml·mmHg⁻¹·kg⁻¹ body wt (80), which corresponds to less than 1% of the total vascular compliance.

Regional partition of the overall compliance is difficult because of nonlinearities of the basic vascular pressure-volume relationship, discontinuities in the distribution of venous pressures, and the inaccuracies inherent in *in vivo* measurements of regional blood volumes (138, 223, 277). The potential of volume measurements based on scintigraphic techniques is yet to be fully explored. Pressure measurements during pneumatic compression of the lower half of the body and during upright immersion (81, 189) suggest that the intrathoracic component of the low-pressure system contributes about 50% of total vascular compliance.

Changing from a supine to an upright position increases venous volume of the legs by about 500 ml (110). Most of the translocated volume is contained in the deep intra- and intermuscular leg veins (213, 214). Additional volume, probably 200–300 ml, is transferred to the veins in the buttocks and the pelvic area. It has been thought that the translocated volume is derived principally from the intrathoracic compartment of the low-pressure system (110, 305) because the hydrostatic counterpressure from the abdominal organs tends to minimize postural changes in transmural venous pressures and venous volume in the splanchnic area. Nevertheless reflex-induced splanchnic vasoconstriction (70, 354) probably produces a passive decrease in venous volume. Measurements based on radionuclear techniques suggest a large contribution also from the splanchnic region (220, 221).

Lower-body negative pressure at –40 to –50 mmHg and standing cause similar shifts in blood volume (355), but the distribution patterns within the lower half of the body are probably somewhat different. The primary pressure gradient along the longitudinal axis of the body (created by decrease in ambient pressure below seal) is stepwise rather than linear (as during standing) and is modified by the tissues surrounding the blood vessels. Wolthuis et al. (355) estimated changes in regional blood volumes and found no changes in plasma-bound isotope activity over the upper abdomen during LBNP at –40 mmHg, whereas the mean increase in activity over the foot, calf, thigh, buttock, and pelvis equaled 24%, 54%, 49%, 39%, and 20%. This heterogeneity can be at least partially attributed to regional differences in the ratio between soft and bony tissues.

There is little quantitative information on the acute intravascular volume shifts during head-down tilt. Significant leg volume changes—as large as during orthostasis—occur during the initial hours after a change from supine to 5° head-down tilt (37). The magnitude

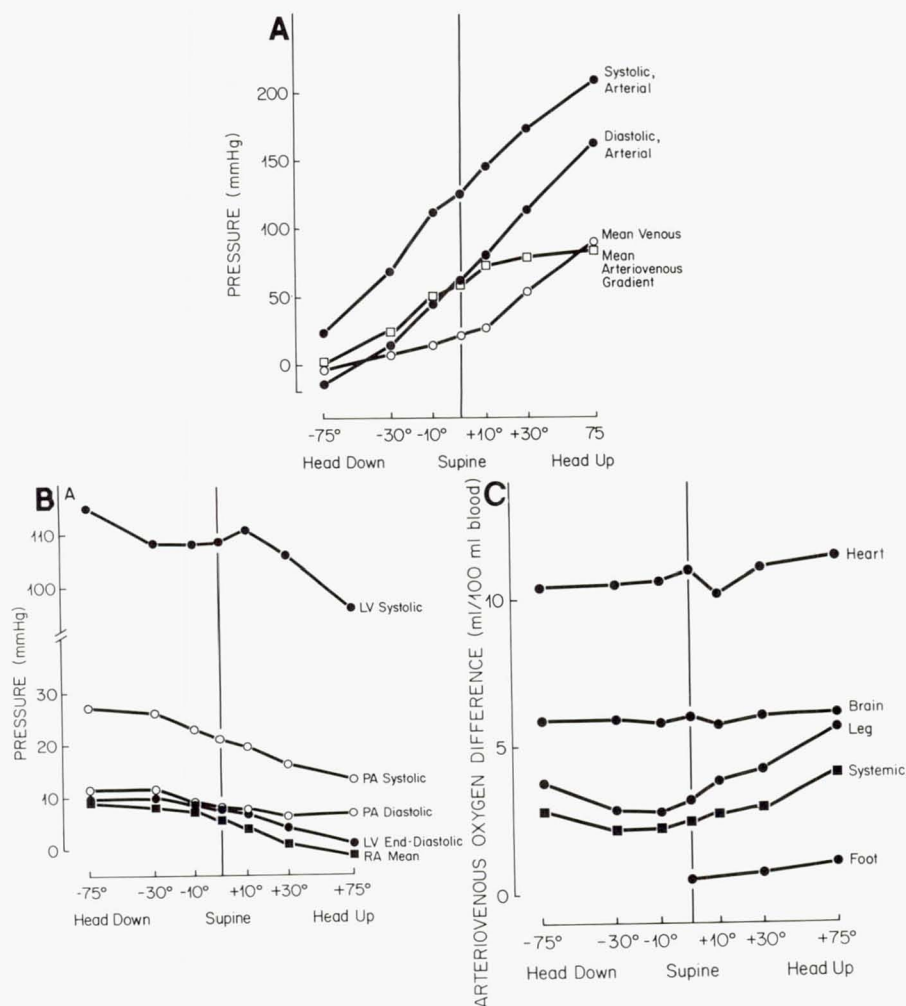


FIG. 5. Responses to graded head-up and head-down tilt in 10 humans. LV, left ventricle; PA, pulmonary artery; RA, right atrium. Intravascular pressures in foot (A) and in central circulation (B), and regional and systemic arteriovenous O_2 differences (C). Angle of tilt (horizontal axis) plotted as sine function to provide linear scale for primary hydrostatic effects of body-position changes. [Based on data from Katkov and Chestukhin (178).]

of the fluid shift during immersion depends on depth and body position. Supine head-out immersion produces no significant acute hemodynamic effects (18) and presumably only insignificant volume shifts. Upright immersion to the level of the diaphragm abolishes the hemodynamic changes that are normally associated with standing. The hemodynamic state is similar to that at rest supine (269). Upright immersion to the level of the neck reverses the normal peripheral orthostatic redistribution of blood volume and increases central blood volume (as determined by indicator-dilution technique) by some 700 ml (4). Farhi and Linnarsson (95) have also demonstrated progressive but nonlinear hemodynamic changes as immersion depth increased.

Intravascular and Intracardiac Pressures

Actual postural pressure changes within the arterial system closely conform to alterations in the hydro-

static pressure component as predicted from the change in vertical distance between the point of pressure measurement and the reference point. Katkov and Chestukhin (178) directly measured pressure at tilt-table positions ranging from +75° head up to -75° head down (see mean data in Fig. 5). In the foot, systolic arterial pressures in these two positional extremes differed by 188 mmHg, which is remarkably close to the expected hydrostatic gradient of 195 mmHg (as calculated from data on distance between right atrium and foot, angle of tilt, and sp gr of blood).

Thin walls and valves make the systemic veins much more complicated than the arteries. Venous pressures in the foot of supine humans are 10–15 mmHg (110, 178). There is a decrease of 2–3 mmHg from the foot to the inferior vena cava at the level immediately below the diaphragm. An additional sharp pressure drop of 2–3 mmHg accompanies entry into the thoracic cavity. A similar gradient is recorded at the thoracic inlet when a catheter is passed from the internal

jugular vein into the superior vena cava (9, 110). The stepwise change in intravascular pressure has the same order of magnitude as the average negative pleural pressure during quiet respiration at rest (232), suggesting that there is little change in transmural venous pressure.

A transition from a supine to an upright position introduces significant nonlinearities. The intravenous pressure distribution (as referred to the level of the right atrium) deviates markedly from the predicted distribution in a simple and uniform closed hydrostatic system, particularly in the regions cephalad to the heart. Even at negative intravascular pressure the intracranial veins are kept open by periosteal adhesions and by parallel hydrostatic pressure changes in the cerebrospinal fluid. Pressures at the level of the thoracic inlet change from a few mmHg positive pressure in the supine position to about 8–10 mmHg negative pressure in the standing position. The neck veins and even the upper part of the superior vena cava collapse in erect normal human subjects, causing a discontinuity in the pressure distribution. The jugular veins form a separate hydrostatic column within which pressures during orthostasis are independent of the right atrial pressure (9, 178).

Deviations from a simple hydrostatic pattern are evident also in the leg veins (Fig. 5). Early postural venous changes in the foot are significantly smaller than the corresponding arterial changes during head-up and head-down tilt. The relationship of the intravenous pressure to the height of the hydrostatic column is curvilinear (178). The data suggest a partial collapse of the leg veins in the head-down position and a significant effect of the venous valves during head-up tilt. However, the valves cannot support the full hydrostatic pressure in the upright position and eventually become incompetent as the veins are distended (151, 182). The collapse of the veins above the level of the heart is also likely to modify lower-body intravenous pressures in the upright position. Part of the venous hydrostatic column is supported by the elastic forces of the intrathoracic tissues as diagrammed in Figure 1C. There is no convincing evidence for venous collapse at the level of the diaphragm (110).

Immediate postural changes in mean atrial and end-diastolic ventricular pressures are essentially linearly related to the hydrostatic component over the entire range from full head-up to head-down tilt with the exception of little or no increase at an angle steeper than -30° head down (see Fig. 5). The difference between the extreme positions is about 10 mmHg. Right and left ventricular filling pressures change in parallel (178). Several investigators (21–24, 119, 120, 328, 329) have reported similar or slightly smaller changes in filling pressures after a transition from supine to sitting or standing (Table 1) and from supine to moderate degrees of head-down tilt (37, 250).

These measurements of intracardiac and central venous pressures may not accurately reflect the postural changes in effective or transmural filling pres-

TABLE 1. *Direct Hemodynamic Measurements in Normal Subjects at Rest*

Parameter	Supine	Sitting	P
Heart rate, beats/min	73 \pm 4	84 \pm 4	<0.001
Pressure, mmHg			
Brachial artery	96 \pm 3	99 \pm 4	
Systolic	130 \pm 5	132 \pm 5	
Diastolic	76 \pm 3	82 \pm 3	<0.05
Pulmonary artery	13 \pm 1	13 \pm 1	
Pulmonary capillary wedge	6 \pm 1	4 \pm 1	<0.001
Left ventricular end diastolic	8 \pm 1	4 \pm 1	<0.001
Stroke index, ml/m ²	50 \pm 5	35 \pm 3	<0.001
Cardiac index, liters \cdot min ⁻¹ \cdot m ⁻²	3.5 \pm 0.3	2.8 \pm 0.2	<0.001

Means \pm SE for 10 sedentary men, age 32–58. [From Thadani and Parker (328).]

ures; the effect of posture on pleural and pericardial pressures should also be considered. Such pressure changes—as discussed in HYDROSTATIC PRESSURE, p. 1025—have been studied in the anesthetized dog (10, 68). The pleura and pericardium behaved much like a simple hydrostatic system. The transmural atrial pressures recorded in the head-up and head-down position did not differ significantly, and cardiac output did not change. The extent to which these findings apply to humans is unknown. Measurements of cardiac dimensions and performance provide overwhelming, if indirect, evidence for significant postural changes in the effective filling pressures in the human. Significant concurrent changes in intrinsic or effective transmural ventricular compliance are unlikely. The different responses in intact humans and anesthetized animals may be due to different locations of the HIP, different extracardiac hydrostatic conditions, reflex activity, or a combination of these factors.

Head-out upright immersion produces an increase in central venous pressure that is proportional to the depth and that averages 10–13 mmHg above the normal standing level (4, 81, 269). Applying LBNP at -40 to -50 mmHg causes a decrease in central venous pressure similar to that during a change from the supine to upright position (37, 167, 244, 250, 355).

Cardiac Dimensions and Pump Performance

The principles governing cardiac pump performance to a large extent determine the cardiovascular responses to changes in posture. Comprehensive analysis of ventricular function includes simultaneous consideration of force, velocity, and fiber length (238). The velocity axis can often be disregarded without serious loss of information in the analysis of the performance of the intact heart. The ventricular pressure-volume relationship (133, 286, 324, 347) is useful for analysis and links muscle mechanics and ventricular function. Contractile state is defined as the maximal tension or pressure that can be developed at any given fiber length or volume. Maximal ventricular pressure is normally a linear function of volume. The amount of shortening or stroke volume that can be achieved from

any given fiber length or volume can be increased only by reducing afterload or enhancing the contractile state. Similarly an increase in the amount of shortening at a given afterload requires either an increased end-diastolic volume or an increased contractile state. Decreased filling pressure, e.g., caused by venous pooling during the transition from the supine to standing position, therefore causes stroke volume to decrease unless compensated for by an increased contractile state or decreased afterload. However, the systolic reserve capacity of the normal human heart is limited. The left ventricular ejection fraction at rest is about 70%. Powerful reflex mechanisms maintain arterial pressure and afterload. A large decrease in preload therefore inevitably decreases stroke volume. Cardiac output can only be maintained by increasing heart rate.

There is ample evidence that postural changes produce significant changes in the dimensions of the heart. Studies based on single or biplane X-rays have demonstrated a decrease in total heart size after a change from the supine to standing position. Although there are fairly large interindividual variations, the average decrease in an adult normal man is 100–200 ml, or 10%–20% of normal total heart volume of up to 500 ml/m² body surface area (152, 176, 245).

More recently, noninvasive imaging methods have provided detailed data on cardiac dimensions and performance in humans. A calibrated nongeometric scintigraphic technique measured left ventricular volumes supine and sitting (Table 2). The relative change in left ventricular end-diastolic volume was of similar magnitude to that in total heart volume. Ejection fraction and end-systolic volume did not change significantly. Thus the postural decrease in stroke volume was primarily caused by a reduced preload. Nixon, Blomqvist, et al. (251) used M-mode echocardiography to study the left ventricle at supine rest, during LBNP at –40 mmHg and head-down tilt at –5°. These interventions significantly decreased the estimated end-diastolic volume 28% during LBNP and increased it 23% during head-down tilt. There were only small changes in ejection fraction with an increase during head-down tilt and a decrease during LBNP but large

effects on stroke volume (+35% and –33%). The heart rate and arterial pressures remained stable, and the velocity of circumferential fiber shortening did not change. These data are also consistent with a marked preload effect on ventricular performance with little change in contractile state. Sandler et al. (292) and Ahmad, Blomqvist, et al. (5) have reported similar results during LBNP.

Parker and Case (259) recently suggested that the normal human left ventricle operates at or near its maximal functional size at rest in the supine position, but data obtained during head-down tilt (250, 251), supine exercise (262), and immersion clearly indicate a diastolic reserve capacity. The increased central venous pressure during upright immersion is associated with a large increase in heart size and stroke volume (4, 18, 40, 81, 95, 195, 269).

Cardiac Output

Thadani and Parker (328) studied hemodynamics in normal sedentary sitting and supine men (see Table 1). Many other investigators (21, 24, 119, 120, 287, 309, 345) have reported similar results. Stroke volume decreases in the upright position by about 33%. The modest increase in heart rate does not fully compensate for the decrease in stroke volume. Therefore cardiac output decreases by about 20%. Mean arterial pressure is maintained and systemic resistance increases in direct proportion to the fall in cardiac output. The systemic arteriovenous oxygen difference also increases. A change from the supine to standing position causes qualitatively similar but slightly larger hemodynamic adjustments than a change from the supine to sitting position (23, 24). Matalon and Farhi (224) demonstrated an inverse linear relationship between cardiac output and the sine of the tilting angle during passive head-up tilt.

Blomqvist et al. (37) found that head-down tilt at –5° does not change arterial pressure or cardiac output but produces an early and transient increase in stroke volume, which causes compensatory bradycardia. Katkov et al. (179) recorded no significant change in right ventricular end-diastolic pressure but a 15% increase in stroke volume after 15 min at –20° head-down tilt. There was a small increase in heart rate, and cardiac output increased by 27%. The lack of change in right ventricular pressure at –20° may seem paradoxical, but experimental data indicate that a steep head-down tilt may decrease filling pressures (110). Upright immersion increases cardiac output by 30% or more, mainly because of an increase in stroke volume without any compensatory bradycardia (4, 18).

Regional Flow

The data on arteriovenous oxygen differences presented in Figure 1 indicate that flow to vital organs, i.e., the brain and the myocardium, remains adequate over the entire range of short-term postural chal-

TABLE 2. Left Ventricular Dimensions and Performance at Rest

Parameter	Supine	Sitting	P
Volume, ml			
End diastolic	107 ± 10	85 ± 6	<0.02
End systolic	34 ± 4	32 ± 5	
Stroke	76 ± 8	55 ± 5	<0.05
Ejection fraction, %	76 ± 2	72 ± 4	
Heart rate, beats/min	71 ± 6	89 ± 5	<0.05
Arterial pressure, mmHg			
Systolic	125 ± 8	125 ± 5	
Diastolic	76 ± 4	84 ± 4	

Means ± SE for 7 normal subjects. [Scintigraphic data from Poliner, Blomqvist, et al. (262), by permission of the American Heart Association, Inc.]

lenges. Changes in posture, however, produce a complex set of regional adjustments.

BRAIN. The oxygen uptake of the brain changes little unless there are functional disturbances of clinical significance (197). Oxygen delivery to the brain is generally well protected during gravitational stresses, perhaps better in humans than in other species (240). Several factors combine to minimize the effects of gravity during postural changes and during acceleration (146), including an efficient autoregulation of blood flow, hydrostatic pressure changes in the cerebrospinal fluid (which are likely to parallel intravascular changes, particularly in arterial pressure), and the architectural and functional characteristics of the intracranial veins. Mean arterial pressure at eye level during tilting or hemorrhage can fall below 30 mmHg before consciousness is seriously impaired and below 25 mmHg before syncope develops (146, 193). The basic efficiency of this system does not necessarily preclude significant postural changes in total cerebral flow (197), but Katkov, Chestukhin, et al. (178, 179) found an essentially stable arteriovenous oxygen difference over the entire range of body positions from -75° to $+75^\circ$ (Fig. 2).

MYOCARDIUM. Changes in arterial pressures, heart rate, contractile state, and ventricular dimensions during posture changes are likely to change myocardial oxygen demand and perfusion. Langou et al. (196) found that head-up tilt at $+30^\circ$ decreased left ventricular end-diastolic diameter, myocardial oxygen uptake, and coronary flow in dogs. The arteriovenous oxygen difference across the myocardium also decreased. Neither the heart rate nor the contractile state changed. Similar detailed data on flow and oxygen demand in the human are unavailable. Katkov and Chestukhin (178) recorded no changes in myocardial oxygen extraction in their human subjects during head-up and head-down tilt.

SKIN AND MUSCLE. The effects of postural changes on blood flow to the resting arm and leg are directionally similar to the changes in overall systemic flow. Increasing degrees of venous pooling during LBNP cause a gradual and sustained reduction of forearm flow (167, 231, 271, 362). The relative importance of muscular and cutaneous vasoconstriction is controversial. Several investigators have concluded that the orthostatic vasoconstrictor response in resting skeletal muscle probably accounts for a major portion of limb flow reduction (8, 12, 128). McNamara (231) found only transient decreases in hand blood flow (primarily cutaneous) during mild degrees of LBNP. More severe LBNP stress caused a sustained flow reduction with no quantitative relation to the degree of venous pooling. Gauer and Thron (110) reviewed several series of experiments and also concluded that the orthostatic vasoconstrictor response in the leg primarily involved skeletal muscle with little or no contribution from the cutaneous circulation. On the other hand Rowell et al.

(282) attributed the decrease in forearm flow during LBNP equally to vasoconstriction in skin and muscle, consistent with significant baroreceptor-mediated effects also on the cutaneous circulation (279).

McNamara et al. (231) noted the similarity between the peripheral responses to head-up tilt and LBNP, which imply that changes in systemic flow and reflex adjustments are more important determinants of limb blood flow than the direct hydrostatic effects. However, hydrostatic effects are evident during extreme degrees of head-down tilt. Katkov and Chestukhin (178) found that blood flow to the foot approached zero in the -75° head-down position.

SPLANCHNIC REGION. The splanchnic bed is a major source from which blood flow and venous volume can be redistributed to other areas during stress. The liver and the splanchnic area contain about 25% of the total blood volume and receive an equally large portion of cardiac output at rest, but they extract less than 20% of the available oxygen (278). Progressive peripheral pooling during LBNP gradually reduces splanchnic flow. Flow rates at -40 mmHg are less than 70% of control (1, 167).

KIDNEYS. The kidneys receive about 20% of the total systemic flow at rest. Orthostatic stress significantly reduces renal plasma flow, as judged from changes in *p*-aminohippuric acid clearance. Head-up tilt at 45° reduces renal flow to about 50% of the supine rate (295, 351). Gilbert et al. (113) reported a 27% reduction in effective renal plasma flow during LBNP at -60 mmHg. In contrast the increased cardiac output during upright immersion does not consistently change renal hemodynamics (87, 88).

Postural Effects on Hemodynamic Responses to Exercise

Many of the postural hemodynamic effects that are evident at rest persist during exercise. Cardiac output is 1–2 liters/min higher in the supine than in the sitting position at rest and during dynamic exercise involving large muscle groups, e.g., bicycle exercise (21–24, 85, 328, 329). Body position does not affect the slope of the normal linear relationship between oxygen uptake and cardiac output. An increase in oxygen uptake of 1 liter/min is associated with an increase in cardiac output of about 6 liters/min in both positions. Thus the systemic arteriovenous oxygen difference is wider in the upright than in the supine position. Total systemic resistance is higher in the upright position at rest and during low-level submaximal exercise, but the differences are abolished during heavy submaximal work (22).

Stroke volume at rest is significantly larger and heart rate is lower in the supine position, but transition from rest to exercise causes a larger stroke volume increase in the upright position (21–24, 328, 345). Heavy leg exercise tends to abolish the positional stroke volume differences. The postural effects tend to

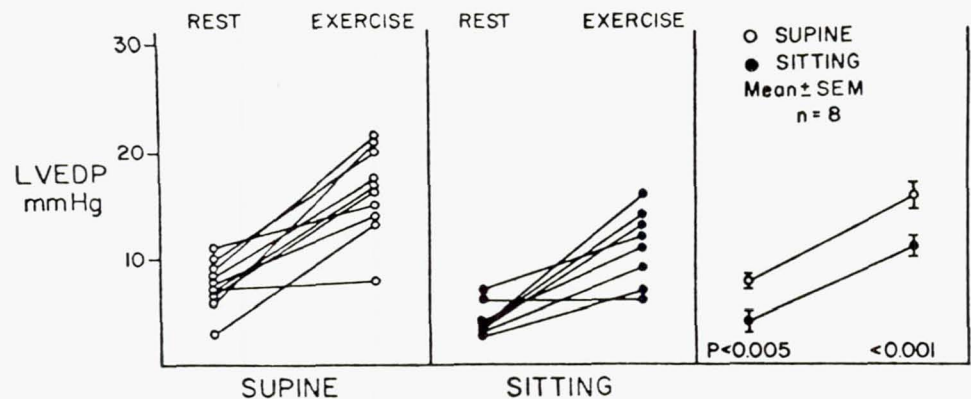


FIG. 6. Normal individual and group mean values of left ventricular end-diastolic pressure (LVEDP) in supine and sitting positions at rest and during exercise. [From Thadani and Parker (328).]

be larger during dynamic exercise with small muscle groups; e.g., arm exercise and stroke volumes are different also at maximal work levels. Relative tachycardia maintains a normal slope of the relationship between oxygen uptake and cardiac output, but absolute flows are 1–2 liters/min below supine levels also during arm exercise (22).

Right and left ventricular filling pressures are higher at rest in the supine position. According to most (85, 119, 120, 328) but not all (117, 276) investigators, exercise in either position progressively increases left ventricular filling pressures, but pressures remain higher in the supine position (Fig. 6). Mean right atrial and right ventricular end-diastolic pressures as referred to the atmosphere tend to remain unchanged or actually decline during exercise (22, 85). Mean pleural pressures, however, become increasingly negative with increasing ventilation and may average -10 mmHg during heavy exercise (232). This means that both right- and left-sided effective filling pressures probably increase during exercise but that the changes are larger in the left ventricle.

As discussed earlier in this section there is ample evidence that at rest left ventricular diastolic volume is larger in the supine than in the upright position. The increase in stroke volume that occurs between rest and exercise in the upright position is associated with an increase in total heart size and left ventricular end-diastolic volume (69, 152, 285). There is general agreement that end-systolic volume decreases during exercise in both body positions (46, 69, 117). The role of the Starling mechanism during exercise in the supine position is controversial (46, 69, 117, 296, 311, 348), however, perhaps because of methodological limitations. Poliner, Blomqvist, et al. (262), who used a quantitative nongeometric scintigraphic method to circumvent many of the problems inherent in echocardiography, contrast ventriculography, and the use of epicardial markers, found a significant increase in end-diastolic volume during supine exercise. Left ventricular end-diastolic volumes were higher in the supine position at rest as well as during submaximal and

TABLE 3. Hemodynamic Effects of Acceleration

Acceleration	Cardiac Output	Heart Rate	Stroke Volume	Mean Arterial Pressure
G_z				
+2	+7	+14	-24	+9
+3	-18	+35	-37	+21
+4	-22	+56	-49	+27
G_x				
+2	-12	-2	-10	+11
+3.5	+9	+17	-7	+18
+5	+27	+40	-8	+25

Effects of head-to-foot ($+G_z$) and transverse gravitational force ($+G_x$) acceleration in 6 relaxed human volunteers. Values are % change from control. [Data from Lindberg et al. (207, 208).]

maximal exercise. End-systolic volume was slightly smaller in the upright position, and maximal stroke volumes were similar (Table 3; Fig. 7). These data are consistent with a combined Starling effect and increased contractile state during exercise, both supine and upright.

The lower systemic arteriovenous oxygen difference during exercise in the supine position indicates a higher perfusion rate of inactive tissue. Blood flow in working limbs is more complex. During leg exercise limb flows are higher, and after exercise they are lower in the upright than in the supine position (98, 99, 206). Greenleaf et al. (128) published exercise data that partially disagree, but their flow data were obtained immediately after exercise. Folkow et al. (98, 99) attributed the higher flow to a higher driving pressure in the upright position. A large hydrostatic component affects intravascular pressures in a dependent limb, but the reduction in venous pressure due to skeletal muscle activity is probably particularly prominent there. The arteriovenous pressure gradient and the perfusion pressure therefore tend to be greater when the working limb is dependent rather than at the level of the heart. Lind et al. (206) studied isometric exercise and found no postural effects on strength, arterial and right atrial pressures, or heart rate, but forearm flow increased and endurance improved in the upright po-

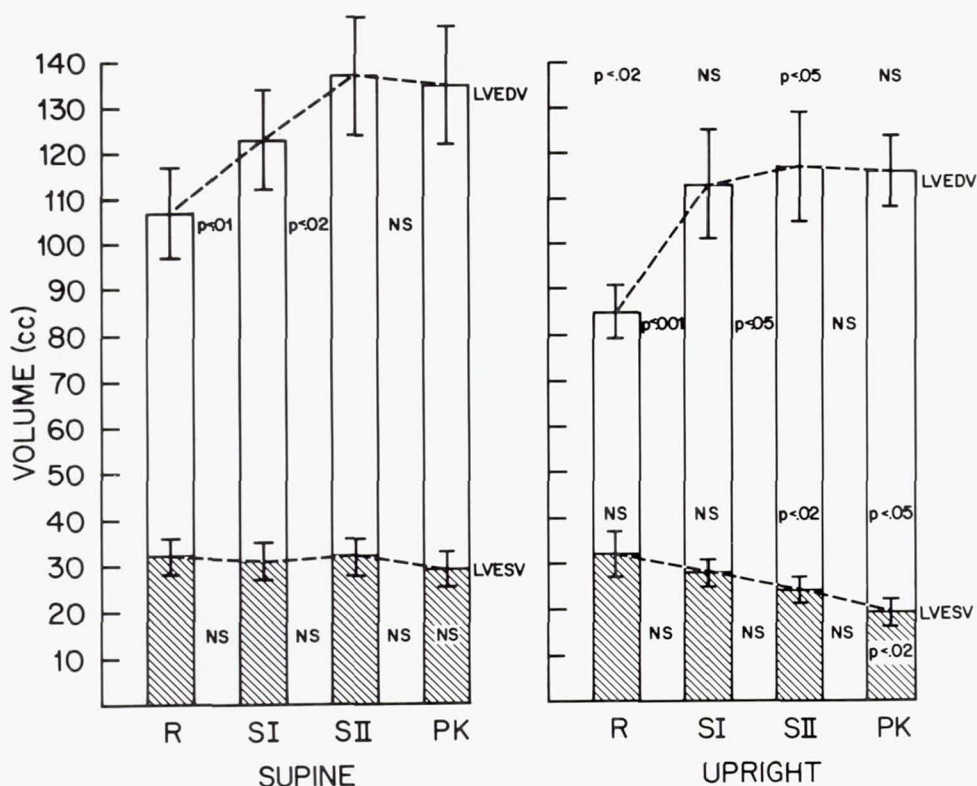


FIG. 7. Left ventricular end-systolic volume (LVESV; shaded portion), LVEDV (means \pm SE; tops of bars), and LV stroke volume (clear portion) at rest (R) and during 3 levels of exercise (SI, SII, PK). Above bars of right panel, P values for corresponding upright and supine measurements of LVEDV at each work load; P values above LVESV data for corresponding supine measurements of LVESV; P values between adjacent bars for change between progressive work loads; P values in small boxes of peak exercise (PK) bars for change from R to PK for LVESV in each position. Between R and PK, LVEDV also increased significantly in both positions ($P < 0.001$ supine, $P < 0.02$ upright). SI, low-level work (300 kpm/min); SII, intermediate-level work (600–750 kpm/min). [From Poliner, Blomqvist, et al. (262), by permission of the American Heart Association, Inc.]

sition. No muscle pump is active during isometric exercise, and the position of the arm relative to the heart was similar in both body positions. These results are consistent with important neural rather than hydrostatic mechanisms. Lind et al. (206) discounted activation of the low-pressure baroreceptor as a possible factor affecting peripheral flow. However, other investigators (2, 281) have found evidence for interaction during exercise between impulses originating in muscle receptors and in cardiopulmonary and carotid baroreceptors. The upright position apparently modifies the inhibitory drive generated by the baroreceptors (2). Bonde-Petersen et al. (41) showed larger systemic pressor responses to isometric knee extension and handgrip in the upright than in the supine position. The apparent disagreement between the absence of right atrial pressure changes reported by Lind et al. (206) and the conclusions of Abboud et al. (2) may be resolved by postulating significant changes in left-sided atrial and ventricular pressures during exercise.

There are no direct data on postural effects of myocardial blood flow during exercise in humans. However, patients with limited flow caused by coro-

nary disease provide evidence for a more favorable relationship between total body and myocardial work in the upright than in the supine position (196, 201, 329). Patients with effort-induced angina pectoris have a highly reproducible threshold of myocardial ischemia, i.e., the point at which myocardial oxygen demand exceeds the supply. The product of heart rate and systolic blood pressure reliably estimates myocardial oxygen demand if there are no major variations in left ventricular volume. The rate-pressure product at the onset of the angina pectoris is higher in the upright than in the supine position, probably because left ventricular filling pressures and volumes are lower (329). Wall tension and myocardial oxygen demand at any given left ventricular pressure are therefore higher during supine exercise.

Dynamic Responses to Posture Changes

REGULATORY MECHANISMS. Gauer argued forcefully that in humans the upright position is the normal reference and that basic vascular pressure-volume characteristics are regulated to provide optimal tissue

perfusion in the upright position (110). Nevertheless the supine position continues to serve as the basal or control state in most human hemodynamic studies. Few investigators have concerned themselves with the immediate circulatory adjustments to a change from a standing or sitting to a lying position. Careful study would probably reveal hysteresis; however, for the purposes of this discussion it is assumed that the hemodynamic responses to the supine-standing and standing-supine transitions are mirror images.

Multiple mechanisms determine the dynamic responses to changes in posture, including neurogenic control mechanisms and the functional characteristics of the veins. The redistribution of intravascular fluid produces reflex responses that primarily involve three different sets of receptors (49, 79, 188, 219, 257, 298, 333, 334): 1) carotid and aortic mechanoreceptors, 2) cardiopulmonary mechanoreceptors, and 3) hypothalamic osmoreceptors and angiotensin II receptors. The third group of receptors interacts with the first two in the control of blood volume and is a less important determinant of the acute responses. Angiotensin II release can stimulate the central nervous system and augment sympathetic vaso- and venoconstriction, but the direct and indirect hemodynamic effects are physiologically important only in the sodium-depleted state (97, 298). Activating the cardiopulmonary mechanoreceptors by distension inhibits both antidiuretic hormone (ADH) and renin-aldosterone and produces a diuresis (87, 89, 90, 107).

Carotid and aortic mechanoreceptors. The mechanoreceptors in the walls of the carotid sinus and the aortic arch are stretch receptors. The degree of deformation and the rate of impulse flow are functions of transmural pressure. Afferent impulses travel in rapidly conducting myelinated and slowly conducting unmyelinated nerves with the glossopharyngeal nerve and the vagus to the cardiovascular centers. Efferent fibers reach the sinus and atrioventricular nodes, the ventricles, veins, and arterioles by vagal and spinal cord pathways. A fall in arterial pressure decreases afferent nerve activity and releases inhibitory activity in the cardiovascular centers. This results in increased heart rate and contractility, vaso- and venoconstriction, and reduced blood flow to the skin and skeletal muscle, the kidney, and the splanchnic region. Increased metabolic demands increase myocardial flow.

Cardiopulmonary mechanoreceptors. At least two sets of mechanoreceptors respond to changes in the intracardiac pressures (see chapter by Bishop et al. in this *Handbook* and refs. 49, 76, 79, 209, 218, 257, 333, 334): 1) discrete endocardial receptors at the junctions of the superior and inferior vena cava with the right atrium and the pulmonary veins with the left atrium, which are connected to the cardiovascular center by myelinated vagal fibers and 2) a diffuse receptor network of unmyelinated vagal fibers (C fibers) connecting all chambers of the heart with the cardiovascular centers. A similarly diffuse network of receptors with

afferent sympathetic spinal cord fibers form a third set. The functions of the members of this group are largely unknown although some are probably pain receptors.

Activation by deformation of both discrete and diffuse receptors affects renal sympathetic drive (decrease) and renal blood flow (increase) identically, but the heart-rate effects are opposite. Increased stretch of the discrete venoatrial receptors causes tachycardia; increased stretch of the diffuse receptors (and of the carotid sinus/aortic arch receptors) causes bradycardia. The left ventricular receptors increase their inhibitory activity whenever cardiac diastolic filling is augmented, whereas dilating the right ventricle has little effect (255, 334). Reciprocal variations in atrial and ventricular/arterial pressures, e.g., high mean atrial and diastolic ventricular pressures and low arterial pressures, as in severe congestive heart failure, tend to minimize the systemic impact of impulses from the combined receptor system (76).

Local reflexes. Local reflex mechanisms may also contribute to the response to orthostatic stress. Henriksen (143, 144) has demonstrated vasoconstriction with decreased limb blood flow in response to local venous distension. Blocking studies indicated that the vasoconstriction was mediated by a local (axonal) sympathetic reflex mechanism. Activation of venous afferent fibers producing reflex-induced leg muscle activity may also counteract postural pooling (330, 331).

Regulation of venous system. Passive and active control mechanisms combine to regulate central venous pressure and the regional distribution of venous volume. Passive volume changes, which dominate in the pulmonary vasculature, are a function of changes in the effective transmural distending pressure, i.e., the difference between intravenous pressure and tissue pressure. Arterial pressure and arteriolar resistance, the pressure in the right atrium and central veins, and the hydrostatic load in turn determine venous pressure. The downstream pressure gradients within the venous system (disregarding hydrostatic pressures) are small, 10 mmHg or less. Therefore the venous component of total peripheral resistance is small and arteriolar pressure is the major determinant of distending pressure and, indirectly, of venous volume. Arteriolar dilatation increases venous pressure and volume. Vasoconstriction has the opposite effect and displaces venous volume centrally (277).

Cardiovascular reflexes and circulating catecholamines mediate active changes in venous capacity. The level of α -adrenergic stimulation is the primary control mechanism. The basic muscle length-tension relationship, which applies also to the venous wall, makes active regulation more effective. A distended vein contracts more effectively when stimulated than a nearly empty vessel (7, 254, 297, 298). Volume changes at low venous pressures are primarily passive, whereas active venomotor mechanisms dominate at

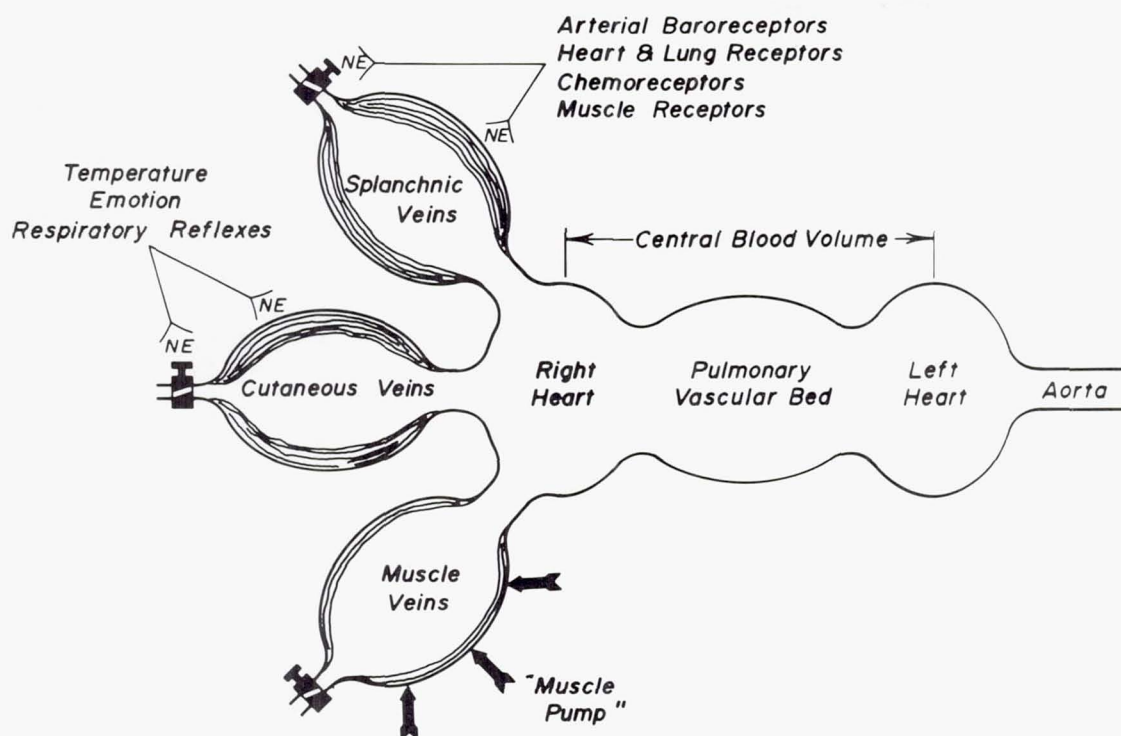


FIG. 8. Control of central blood volume and cardiac filling pressure by systemic veins. Contracting smooth muscle cells of venous wall, splanchnic veins in particular, actively changes filling of heart. Cutaneous veins mainly react to changes in temperature. Any change in distending pressure, whether due to gravity or changes in arteriolar resistance, passively changes capacity. NE, norepinephrine. [From *The Human Cardiovascular System*, 1979, by Shepherd and Vanhoutte (298), by permission of Raven Press, NY.]

high distending pressures (254). The importance of true autoregulatory changes (Bayliss effect) with increased myogenic activity in response to increased intravascular pressure is controversial (234). The opposite effect, i.e., relaxation after a prolonged increase in distending pressure (delayed compliance or viscoelastic creep), may be significant (358). By decreasing venous resistance due to relaxation of sphincters, e.g., in the hepatic vein, β -adrenergic stimulation may centrally displace venous volume (163).

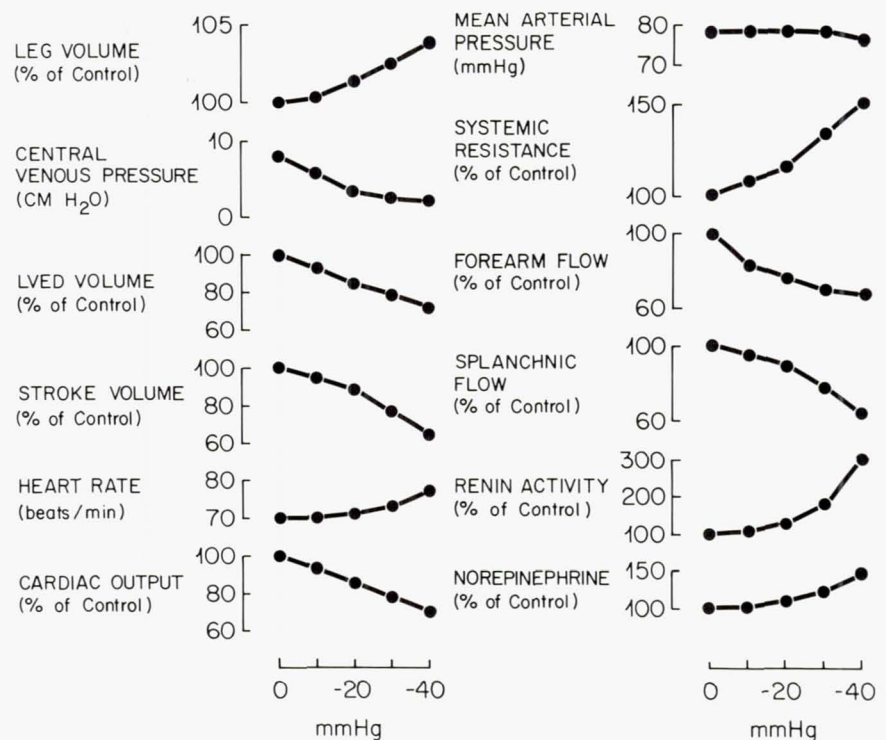
Veins react differently to active stimulation (Fig. 8). Muscle veins respond weakly, if at all, to neural stimulation, including baroreceptor-mediated reflexes. The capacitance of the cutaneous veins is primarily determined by the thermoregulatory stimuli. They dilate when temperature is increased, whereas deep limb veins dilate when perfused with cold blood (297). The splanchnic veins dilate when carotid and cardiopulmonary receptors are stimulated and contract when the muscle receptors (e.g., during exercise) and the chemoreceptors of the carotid body are activated (138, 297). Whether active venoconstriction is a determinant of acute postural hemodynamic responses is questionable. Gauer and Thron (110) thought that venoconstriction was not significant during orthostasis. Samueloff et al. (289) have shown only a weak and transient constriction of cutaneous veins at the onset

of LBNP at -60 mmHg. However, passive changes in venous volume caused by the splanchnic and peripheral vasoconstriction are probably significant (48, 70, 354).

SYSTEMIC RESPONSES. Figure 9 shows the principal cardiovascular responses to LBNP. As discussed in *LOWER-BODY NEGATIVE PRESSURE*, p. 1029, and *SKIN AND MUSCLE*, p. 1035, there is evidence that the responses to LBNP, standing, and head-up tilt $\geq 70^\circ$ are qualitatively and quantitatively similar. The diagram comprises data from several series (5, 96, 100–102, 166, 167, 241–244, 250, 251, 266, 267, 280, 362). Linear interpolation compensated for differences between protocols for stepwise application of LBNP and gave uniform intermediate data points.

The degree of venous pooling is linearly related to the negative pressure. Left ventricular end-diastolic volume and stroke volume also decrease in a linear fashion to a level 25%–50% below that at rest. Cardiac output decreases 20%–40% (5, 100–102, 250, 266, 267). Initially heart rate and arterial pressure do not change. Peripheral and splanchnic arteriolar constriction compensate for the decrease in systemic flow. Progressive peripheral venous pooling eventually causes tachycardia, and the heart rate changes are closely linked to changes in arterial pulse pressure (167). The myocar-

FIG. 9. Principal features of human response to progressive lower-body negative pressure.



dial contractile state does not change until tachycardia develops. Thus the primary cardiac response is a negative Starling effect. An increase in plasma renin is apparent after about 2 min, and there are parallel changes in plasma norepinephrine (F. A. Gaffney, unpublished observations).

The combined data are consistent with a generalized vasoconstriction in response to decreased intracardiac pressures and volumes mediated by a decreased level of stimulation of cardiopulmonary receptors and reflex α -adrenergic stimulation. Increased sympathetic drive produces decreased renal blood flow and a release of renin. Progressive pooling with decreasing stroke volume and cardiac output eventually causes a fall in arterial pressure with compensatory tachycardia, increased contractility, and further vasoconstriction mediated by the arterial baroreceptors. An increase in plasma catecholamines reflects sympathetic activation. Ewing et al. (94) thought that primarily vagal withdrawal mediated the early orthostatic heart-rate response, but data on autonomic blockade during head-up tilt or LBNP indicate that the true reflex response consists of a combined α - and β -adrenergic activation and vagal withdrawal (34-36, 211, 242, 293, 349). β -Adrenergic blockade attenuates the heart-rate response, and vagal withdrawal only partially compensates. The vasoconstrictor response, however, is enhanced. The relative contribution of β -adrenergic stimulation and vagal withdrawal to cardioacceleration may vary with experimental conditions. Atropine has little or no effect on orthostatic tolerance.

The sympathetic activation is clearly nonuniform. Vasoconstriction is manifest before heart rate or con-

tractility is measurably affected. Forearm flow and conductance decrease significantly before splanchnic flow is affected (1, 167). The differential effects on limb flow and splanchnic flow are probably related to a predominance of low-pressure baroreceptor effects at mild degrees of LBNP and increasing effects on the carotid sinus with progressive pooling and decreasing arterial pressures and pulse pressure (1, 167). Non-uniform regional vasoconstrictor responses may also be related to differential effects of changes in the impulse flow from ventricular and arterial baroreceptors and to unequal levels of basal vasoconstrictor activity (350).

The baroreceptor-mediated vasoconstriction in skin and muscle is apparent, although attenuated, even when opposed by thermoregulatory vasodilatory reflexes (166). Thus arterial and venous responses to a hot environment combine to increase the sensitivity to orthostatic stress. Conversely, cooling the skin attenuates the cardiovascular effects of LBNP (266, 267). The combined effects of a hot environment on resistance and capacitance vessels also impair ventricular filling during upright exercise (166, 279).

The involvement of multiple sets of the receptors may explain the relative lack of compensatory tachycardia when venous pooling has caused large decreases in stroke volume and cardiac output and falling arterial mean and pulse pressures. Presyncopal heart rates rarely exceed 130 beats/min; i.e., they remain at least 40 beats below normal maximal heart rate. This relatively slow rate may be the result of opposing chronotropic drives originating in the venoatrial and the cardiac and arterial receptors. This dissociation may

have a role in hypovolemic syncope. Furthermore a reduction of ventricular dimensions during the presyncope state may progress until the end-systolic volume approaches zero and until endocardial deformation receptors are stimulated by compression during systole rather than by high wall tension, as in obstruction of the left ventricular outflow tract (222). Bradycardia and vasodilatation would then be produced by a Bezold-Jarisch-type reflex (92). Echocardiographic studies (5, 251, 292) support this mechanism by demonstrating a trend toward smaller end-systolic volumes with progressive venous pooling. The unstable autonomic state during presyncope with large oscillations in heart rate and arterial pressure (157) may reflect variations in the balance between opposing drives from atrial, ventricular, and arterial receptors.

CARDIOVASCULAR ADAPTATION TO PROLONGED BED REST, ZERO GRAVITY, AND RELATED CONDITIONS

Experimental Conditions

The human responses to prolonged exposure to zero gravity, bed rest, and water immersion have many important features in common. The hydrostatic intra- and extravascular pressure gradients that are normally present in the upright position are abolished or minimized. This causes a central or cephalad fluid shift that initiates a complex series of adaptive changes in several organ systems. After adaptation, reexposure to normal gravitational forces produces signs of orthostatic intolerance.

BED REST. More information is available on prolonged bed rest than on other hypogravic conditions. A recent summary (249) lists more than 500 American, European, and Russian bed-rest studies performed between 1921 and 1978. Early experiments concentrated on the metabolic effects of bed rest and immobilization and documented increased nitrogen excretion (54), calcium loss (6, 71), and decreased glucose tolerance (38). Clinically important side effects of bed rest, including loss of muscle mass and strength, cardiovascular deconditioning, and an increased risk of venous thromboembolism, were recognized during the early 1940s (75, 141, 203, 204). Taylor et al. (326, 327) and Dietrick et al. (74) described the principal effects on body-fluid distribution and cardiovascular function, i.e., decreased blood volume, orthostatic intolerance, and decreased exercise capacity in the upright position. The establishment of the US manned space-flight program in 1958 and extensive use of bed rest as a model of weightlessness yielded many studies during the 1960s.

HEAD-DOWN TILT. Russian investigators introduced bed rest with head-down (antiorthostatic) tilt, usually at -4° to -6° , as a more effective technique of simulating zero gravity than horizontal bed rest (177).

Several Russian and American studies (37, 65, 177, 180, 250, 302, 303, 343) have demonstrated that the effects of head-down tilt are qualitatively similar to those of bed rest except that the adaptation is accelerated. During short-term experiments head-down tilt produces a greater degree of cardiovascular deconditioning than horizontal bed rest of equal duration. One study (158) has failed to demonstrate any difference between the two modes. In general the comparisons of the effects of space flight and head-down tilt support the use of tilt as a simulation method for cardiovascular studies (37, 250, 291).

IMMERSION. The immediate effects of water immersion on fluid distribution and hemodynamic conditions are (as discussed in IMMERSION, p. 1030) more powerful than those of bed rest and head-down tilt. Prolonged immersion is logistically difficult, but hemodynamic measurements have been obtained over periods of several hours (4). Immersion periods of up to 56 days have been accomplished by protecting the subjects from water contact by wrapping them in thin plastic sheets (302).

ZERO GRAVITY. Only limited physiological data on the effects of zero gravity were collected during the early phases of the US and USSR manned space-flight programs (261). More extensive studies were made during the Apollo flights of 6–12 days (174), the three Skylab flights of 28–84 days (173), and the two Russian Salyut-6 flights of 96 and 140 days (359).

Data obtained during actual space flights are, of course, crucial in the study of the effects of gravity on the cardiovascular system, but the number of subjects that can be studied in space and the range of methods are, although rapidly expanding, still very limited. Ground-based simulation methods may closely reproduce many important aspects of weightlessness, but residual hydrostatic gradients are always present. The earth's gravitational field can be cancelled in aircrafts following parabolic or Keplerian flight patterns but only for periods of less than 45 s (291). However, there is no conclusive evidence that zero gravity has any unique or specific effects on the cardiovascular system that cannot be reproduced at least in kind during earthbound simulation studies. Similar considerations apply to the musculoskeletal system, but the effects of zero gravity on the vestibular system are probably specific. Motion sickness is common during the initial week of space flight (123). Provocative tests at normal gravity cannot predict susceptibility. Unusual movements and an altered relation between vestibular and visual inputs during space flight are thought to be major pathophysiological factors (123).

Age, sex, and physical fitness may affect the response to zero gravity, but no conclusive information is available. Astronauts and cosmonauts with few exceptions have been male, age 35–45, and physically fit. Most bed-rest studies have been performed in normal men in their twenties, but the NASA-Ames Research

Center has during the past few years conducted a series of studies in women, 20–35 yr old, which in general fail to reveal any significant sex differences (116, 130, 247). Convertino, Hung, and co-workers (66, 156) recently completed a 14-day bed-rest study in 50-yr-old men. The responses, including the degree of post-bed-rest cardiovascular deconditioning, were qualitatively and quantitatively similar to those of younger men. Corresponding findings have also been made during and after head-down tilt (F. A. Gaffney and C. G. Blomqvist, unpublished observations).

The role of physical fitness in the response to bed rest and related conditions is complex. Exercise has been used extensively to counteract deconditioning. Very fit subjects on the other hand appear to recover more slowly from prolonged bed rest than sedentary subjects if pre-bed-rest exercise performance is the basis for comparison (287). Previously fit, deconditioned individuals are nevertheless likely to outperform sedentary subjects. Athletes have often been considered more sensitive to short- and long-term orthostatic stress than sedentary subjects (187), but objective comparisons of athletes and nonathletes have variable results (47, 246, 310).

Body Composition

Bed rest, space flight, and water immersion initially decrease body weight 1–2 kg because of a loss of extracellular fluid. The magnitude of the initial weight loss closely corresponds to the cephalad fluid shift (37, 202, 250, 335, 336, 353).

There is a brief and transient phase of plasma volume expansion because of transfer of fluid from the extravascular compartment, but a decrease in plasma volume accounts for about one-third of the early weight loss (84, 129, 137, 226–228, 332). The fluid-related weight loss occurs within 3–4 days during bed rest (127, 340) and within 1–2 days during head-down

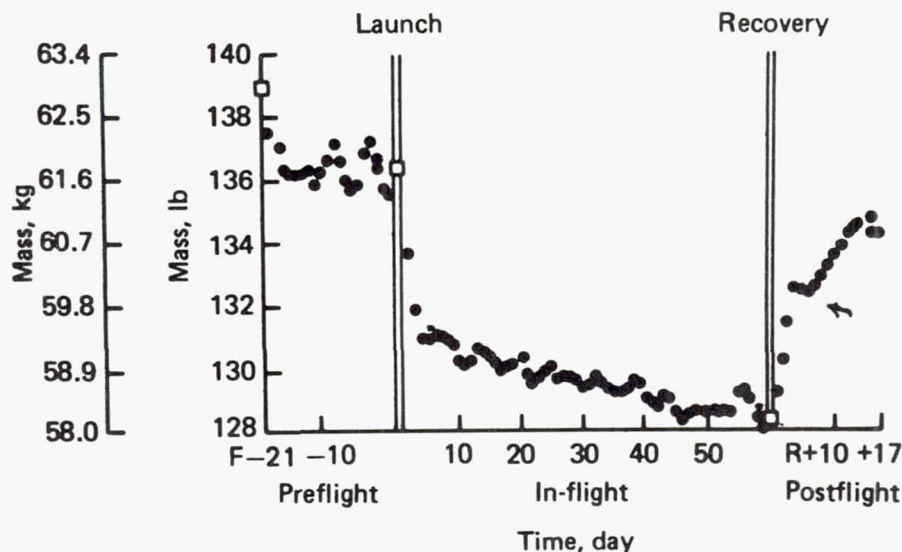
tilt and space flight (37, 150, 250, 261, 335, 336). The effect of water immersion is more rapid. A change of more than 1 kg has been recorded after a 6-h period of immersion (90).

The average total weight loss during flights of 28–84 days in the nine Skylab crew members was 3 kg, corresponding to 4% of total body weight (335, 336). Changes within the initial 3 days in space accounted for ~50% of the total loss (Fig. 10). Leach et al. (199) identified three components of the changes in total body mass: 1) early obligatory water loss of about a liter as a consequence of a central fluid shift, 2) gradual loss of muscle mass due to disuse atrophy, and 3) variable change in the amount of body fat due to a caloric deficit. Early potassium losses were significant during the Apollo missions and may have contributed to the development of transient arrhythmias (20). Potassium loss with hypokalemia has also been observed during short-term simulation experiments (LBPP) in primates (239). Data from later flights, however, suggest that muscle atrophy and loss of body fat account for most observed overall losses of potassium, nitrogen, and protein (199, 200, 202); arrhythmias have not been a significant problem. A calcium loss is physiologically important but numerically represents only a small fraction of the total weight loss.

Disuse atrophy of skeletal muscle can cause secondary changes of cardiovascular function by affecting muscle tone, venous pooling, and the response to exercise. Disuse atrophy preferentially affects red fibers in general (42) and postural muscles, which contain a high proportion of red or slow-twitch fibers (288). The atrophy is reversible; muscle mass is restored relatively rapidly (~2 wk) after experimental immobilization, but postural muscles (e.g., soleus) may require 3–4 mo to regain full strength (42). Similar observations have been made in rats after space flight (60, 256, 325).

Loss of calcium is consistent in the response to bed rest and weightlessness. Prolonged bed rest has pro-

FIG. 10. Body-mass measurement of Skylab-3 scientist pilot at 0 *g* with spring-loaded oscillating system in which frequency of oscillation was proportional to mass. [From Thornton and Ord (336).]



duced losses at a rate of 0.5% of total body calcium per month. The process is progressive, and the rate tends to accelerate (74, 77, 287). The calcium loss appears to be more severe during weightlessness than during bed rest. Balance studies during the Skylab flights demonstrated combined urinary and fecal losses at a rate as high as 2.5% of total body calcium per month (265). The threshold for clinically important osteoporosis is a cumulative loss of about 20%.

A balance between calcium uptake and loss is restored shortly after the return to normal gravity. Nevertheless prolonged exposure to weightlessness may cause irreversible skeletal damage. Urolithiasis is also a potential complication. Because it lacks effective countermeasures calcium loss is placed along with radiation exposure (337) as the other principal biological factor limiting the safe duration of space flight.

Calcium loss is almost certainly triggered by the absence or attenuation of the forces that normally support the body weight, i.e., compression forces acting on bone and shearing and tensile forces acting on periosteal surfaces. Bed-rest studies document failure of vigorous exercise in the supine position to prevent the calcium loss, whereas 3 h of quiet standing each day is effective (77, 164). Young patients immobilized with multiple fractures occasionally develop severe calcium loss from bone and secondary systemic manifestations of hypercalcemia, e.g., massive renal calcification with depressed function and hypertension. Dietary and pharmacological measures are ineffective (6, 64), but mobilization promptly normalizes calcium metabolism.

The exact cellular mechanisms mediating the calcium loss and the net absorption of bone during prolonged bed rest and space flight are unknown. Blood levels of calcium are usually only slightly elevated, and plasma levels of parathyroid hormone are low. No direct links to disturbances of cardiovascular function have been established.

Blood Volume

Plasma volume has been measured by similar techniques in many bed-rest studies, often by multiple determinations. The mass of red cells changes little during the initial 4 wk (127), and changes in plasma volume approximate the changes in total blood volume. The data from several studies with bed-rest periods of 6 h to 30 days (59, 126, 127, 170, 287, 314, 316, 338, 340-342) indicate significant plasma volume decreases within 6 h. The plasma volume contraction is progressive during the first 3 days, but then a plateau is reached at an average loss level of 350 ml or about 12%. Greenleaf et al. (126) performed a similar analysis of the literature and derived a nonlinear regression equation indicating a progressive loss through 60-80 days. Their estimate was heavily influenced by data from a single long-term study (77) with very large losses, 600-1,000 ml at a duration of 70-200

days. However, a similar long-term Russian study (258) showed a loss of 12% after 120 days, a value equal to the typical loss after 4-30 days.

A blood volume loss of 5%-15% is also a feature of space flight and head-down tilt. Prolonged space flight produces a combined loss of red cell mass and plasma volume. Data from early US flights demonstrated loss of red cell mass also after relatively short missions, but exposure to a hypobaric but hyperoxic atmosphere may have affected these results. A significant combined loss of plasma volume (-12.5%) and red cell mass (-11.1%) was observed after the normoxic Skylab flights and also after the 96- and 140-day Salyut-6 flights. The etiology of the decrease in red cell mass is not known, but the mechanism is bone marrow inhibition rather than increased destruction (171, 359). The interaction between the initial decrease in plasma volume and the effects on red cell mass during space flight and bed rest of very long duration need further exploration. The initial plasma volume loss with an increase in hematocrit and oxygen-carrying capacity may inhibit erythropoietin production (171).

Head-down tilt at moderate angles, -4° to -6° , and water immersion also cause blood volume losses of 350-500 ml. Most of these changes occur within 6 h (37, 108, 250). Immobilization in the upright position (i.e., chair rest), combined with horizontal bed rest during sleep, is associated with a reduction in plasma volume. The time course appears to differ from that during bed rest, however, implying a different mechanism. Lamb et al. (190, 192) found no significant changes after chair-rest periods of 4-8 days but a mean decrease of 376 ml after 10 days.

Cardiovascular Function

MAXIMAL OXYGEN UPTAKE. Maximal oxygen uptake as measured during a dynamic leg exercise is an important reference point in cardiovascular physiology. Pulmonary function does not limit uptake in normal subjects (except at high altitude); measurements of the maximal rate of oxygen uptake therefore estimate the overall functional capacity of the cardiovascular system. Cardiac pump function is probably the rate-limiting step under normal conditions, but peripheral mechanisms are likely to become important in many experimental situations. Thus, whereas measurements of maximal oxygen uptake accurately reflect changes in total cardiovascular performance, supplementary data are needed to isolate specific mechanisms.

In normal young men, bed rest of 3-4 wk decreases maximal oxygen uptake (measured during exercise in upright position) 13%-28% (31, 237, 287, 327). Similar changes (-15% after 10 days) have recently been reported in 50-yr-old men (66). Changes during exercise in the supine position in comparable groups are much smaller (66, 67, 112). Data on maximal oxygen uptake after space flight or immersion are not available. Nixon, Blomqvist, et al. (250) reported a decrease of

22% in normal young men after 24 h of head-down tilt at -5° . Lamb et al. (192) found no significant changes in maximal oxygen uptake after prolonged chair rest.

CARDIAC DIMENSIONS AND PERFORMANCE. Adaptation to hypogravic conditions reduces heart size. Saltin, Blomqvist, et al. (287) estimated a significant (-11%) decrease in total heart size in the supine position from biplane roentgenograms in young men after a 3-wk period of bed rest. Radiographic measurements after space flight have consistently demonstrated a decrease in heart size (248). Echocardiography before and after the Skylab-4 flight and two long Salyut-6 flights also showed a decrease in left ventricular end-diastolic diameter (148, 359).

Saltin, Blomqvist, et al. (287) noted that stroke volume was depressed in both the upright and supine positions after bed rest and postulated that prolonged bed rest depressed intrinsic myocardial function. They assumed that any abnormalities exclusively caused by decreased ventricular filling would be abolished in the supine position (25, 26). However, recent echocardiographic and scintigraphic studies have demonstrated decreased preload also in the supine position after adaptation and effectively ruled out any significant changes in contractile performance. Scintigraphic studies by Hung et al. (156) after bed rest showed an increased left ventricular ejection fraction at rest and during exercise both in the supine and upright position. Relative tachycardia was present during upright and supine exercise. The data, derived from a study of 50-yr-old men, indicate that an increased ejection fraction combines with relative tachycardia to compensate for the effects of the decreased end-diastolic left ventricular volume. Head-down tilt (37, 250) also decreases upright and supine stroke volume without changing the contractile state.

Henry et al. (148) found no change in intrinsic cardiac performance after prolonged space flight. They evaluated by echocardiography the ventricular function curves relating left ventricular stroke volume to end-diastolic volume. Two of the three subjects showed a small decrease in estimated left ventricular mass. Russian investigators have recently described ultrastructural myocardial abnormalities affecting the mitochondria and sarcoplasmic reticulum as well as capillaries and venules in rats exposed to zero gravity for 20 days (274). Furthermore the reduced left ventricular size in the Salyut-6 cosmonauts after return to normal gravity was associated with increased left atrial diameter, according to echocardiography (359).

The combined data on cardiac dimensions after bed rest and after space flight strongly suggest a reduction in filling pressures in the postadaptive state. Few direct pressure measurements have been made. Katkov et al. (180) reported no significant changes in supine right ventricular end-diastolic pressure after 5 days of head-down tilt at -4° , but Blomqvist et al. (37) recorded a significant decrease in central venous pressure (from 4.9 to 2.5 cmH₂O) after 24 h of head-

down tilt at -5° . Hemodynamic data (see **SYSTEMIC HEMODYNAMICS**, below) are also consistent with decreased filling pressures after adaptation. On the other hand the early stages of supine bed rest and head-down tilt (37) are probably associated with a transient increase in filling pressures above the levels prevailing in the upright position. Water immersion and head-down tilt raise filling pressures above the normal supine level.

There are no longitudinal data on filling pressures and cardiac output from bed-rest studies. Direct measurements during head-down tilt indicate that the increase in central venous pressure is transient and lasts less than 2 h, whereas the hemodynamic change during immersion persists for several hours with no signs of resolution (4, 37, 81, 250). Because no direct measurements are available the time course and magnitude of changes in filling pressures during space flight can only be guessed. Clinical observations indirectly support a sustained elevation of venous pressures, which may be associated with a hyperkinetic state. Distended neck veins, a puffy facies, and subjective sensations of fullness of the head are consistent features at zero gravity (186, 335). During the Skylab flights this condition persisted over periods of up to 84 days. Yegorov (359) reported a postflight increase in left atrial diameter, consistent with a sustained volume overload similar to conditions during immersion. Studies on supine cardiac dimensions immediately after return to normal gravity, however, clearly indicate an adaptation with decreased ventricular filling. Possibly the distension of the neck veins and the facial edema in space only reflect equilibration of systemic venous pressures. Regional differences in the compliance of the cutaneous vasculature and in tissue-filtration characteristics (186) may combine with an altered regional distribution of venous pressure to produce physical signs of venous congestion in the absence of any sustained elevation of cardiac filling pressures. The Skylab astronauts retained a normal capacity for heavy dynamic leg exercise in space (236); this is strong evidence against any major cardiovascular dysfunction at zero gravity.

Simulation studies provide no conclusive evidence for a sustained hyperkinetic state in space. The magnitude of the central fluid shift and the degree of postadaptive dysfunction are similar after space flight and head-down tilt. A sustained hyperkinetic state with an increase in filling pressures may be a unique product of the hydrostatic conditions that are associated with immersion, and the response to zero gravity may be better approximated by the adaptation to head-down tilt. Immersion causes tissue compression and a significant transthoracic pressure gradient, features that are absent at zero gravity and during head-down tilt.

SYSTEMIC HEMODYNAMICS. The principal effects on systemic hemodynamics of 3 wk of bed rest in normal young men are illustrated in Figure 11. The most

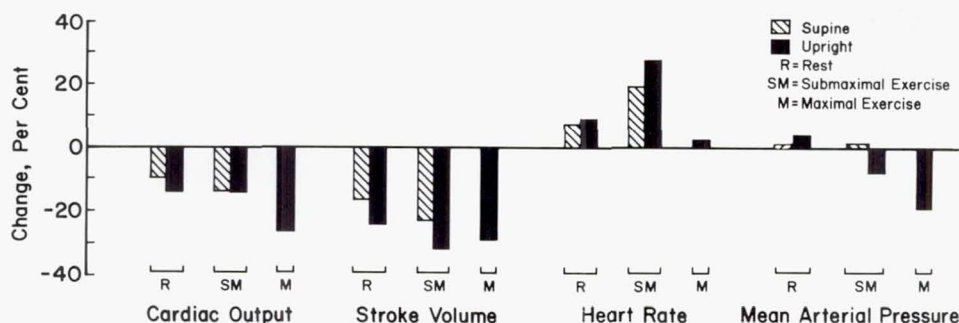


FIG. 11. Hemodynamic effects of 3-wk bed rest on cardiac output, stroke volume, heart rate, and mean arterial pressure supine and upright at rest during exercise in 5 normal young men. Control measurements before bed rest = 100%. [Data from Saltin, Blomqvist, et al. (287).]

important change was a marked reduction in stroke volume at rest and during exercise, particularly in the upright position but also in the supine position. After bed rest a relative tachycardia was noted at rest and during submaximal exercise, but cardiac output was still significantly reduced. Maximal heart rate did not change. The systemic arteriovenous oxygen difference was higher after bed rest at rest and during submaximal exercise but unchanged during maximal work. The maximal oxygen uptake decreased in direct proportion to stroke volume. Mean arterial pressure remained unchanged at rest and during submaximal exercise but was significantly lower at the maximal level. Systemic peripheral resistance was generally higher after bed rest, but the changes were small and nonsignificant.

Hyatt (157) studied a series of 16 subjects before and after a 2-wk bed-rest period. Results obtained at rest and during submaximal exercise in the supine position paralleled the findings by Saltin, Blomqvist, et al. (287). The stroke volume was significantly reduced also in the supine position, particularly during exercise (-20%) and passive head-up tilt. Birkhead et al. (31) have reported similar results. Chobanian et al. (59) also found a markedly reduced stroke volume (-50%) compared to measurements before bed rest during 70° head-up tilt. Nixon, Blomqvist, et al. (37, 250) reported similarly large changes after a 24-h period of head-down tilt at -5°. Stroke volume at rest supine decreased by 22%. Limited observations are available on cardiac output and stroke volume at rest and during exercise after prolonged space flight. Measurements based on the CO₂ rebreathing method were performed in the Skylab crew members after 28-84 days in space. Stroke volume during submaximal exercise decreased by more than 30% compared to preflight data (51, 236). Similar results were obtained after the 2- and 8-day flights of Soyuz 12 and 13 (181).

Extensive heart-rate data are also consistent with a significant stroke volume reduction at rest and during exercise after bed rest and related conditions. Post-bed-rest heart rates during relatively heavy submaximal exercise increased 30 beats/min in the upright and 25 beats/min in the supine position in the study by Saltin, Blomqvist, et al. (287). Others have reported

similar results after prolonged bed rest (31, 66, 157, 327), head-down tilt of short duration (37, 250), and space flight from 6 to 84 days (51, 236, 283). In contrast the in-flight exercise data from the Skylab missions (236) showed little or no change from preflight data. Three of the nine Skylab crew members actually had lower heart rates during exercise in space, suggesting, if anything, an improved exercise capacity at zero gravity.

There is little information on the distribution of cardiac output during and after exposure to hypogravitic conditions. Saltin, Blomqvist, et al. (287) found a normal systemic arteriovenous oxygen difference during maximal exercise consistent with a normal pattern of redistribution of flow to active tissues.

Physical signs and symptoms suggesting cranial venous congestion at zero gravity have raised concerns about the state of the cerebral circulation. However, there is no evidence for any significant impairment of cerebral function in space. Russian studies on the cerebral circulation at zero gravity and during head-down tilt, based on direct measurements of arteriovenous oxygen differences (179) and rheography or impedance plethysmography (359), suggest increased cerebral flow, but the significance of these findings has not been established.

ORTHOSTATIC INTOLERANCE. Many bed-rest studies measuring heart rate and indirect arterial pressure have evaluated changes in orthostatic tolerance. Head-up tilt at 70° for 5-20 min is the most widely used method, but a variety of alternate procedures has also been employed, e.g., LBNP and +G_z centrifugation. The maximal heart-rate difference between supine rest and head-up tilt typically increases from about +25 beats/min before bed rest to +40-+50 beats/min after bed rest (59, 194, 327, 340, 342). Arterial pressures during orthostatic stress are lower after prolonged bed rest, and the incidence of presyncope and syncope increases significantly. Brief exposure to head-down tilt (24 h) and to water immersion (6 h) has produced equally large changes in orthostatic tolerance, as judged from the heart-rate response to LBNP and tilt (37, 228, 250).

The Skylab-4 astronauts showed an average post-

flight increase in heart rate during LBNP at -50 mmHg of 27 beats/min after 89 days in space. In-flight LBNP measurements demonstrated similar changes. Decreased tolerance developed during the first few days without further changes, and toward the end of the flight there was even some improvement (172). The Apollo astronauts (150) had after much shorter exposure to zero gravity a mean heart rate during LBNP at -50 mmHg of 109 beats/min compared to 76 beats/min preflight, i.e., a difference of 33 beats/min.

Countermeasures. Investigators have tested a wide variety of interventions in attempts to prevent the cardiovascular deconditioning induced by bed rest and space flight. Principal approaches include exercise, redistribution of venous volume, and blood volume expansion.

1. Even vigorous exercise in the supine position fails to prevent orthostatic intolerance after bed rest. The effects on exercise capacity in the upright position are variable. A significant decrease is rarely prevented, but the reduction tends to be smaller than in nonexercising controls (29, 30, 45, 56, 194, 237, 323). Most investigators have used dynamic exercise, but static exercise had unexpectedly favorable effects in a study by Stremel et al. (323). Exercise may be a more valuable countermeasure during space flight than during bed rest. The crew members of Skylab 4 performed a large amount of exercise in space and had less cardiovascular dysfunction postflight than the crew members of Skylab 2 and 3, who exercised less vigorously during their shorter missions (172, 236). Current Russian in-flight regimens also include an extensive exercise program (359).

2. Intermittent redistribution of venous volume during bed rest to approximate the normal intravascular pattern during sitting or standing is helpful. Birkhead et al. (29, 30) demonstrated that a daily 3-h standing or 8-h quiet sitting prevented the development of orthostatic intolerance. Prolonged application of LBNP is cumbersome but prevents or minimizes plasma volume loss and orthostatic intolerance (191, 314-316). Other devices, designed to simulate normal hydrostatic gradients, e.g., an elastic reverse-gradient garment, have been effective in short-term (<4 h daily) applications (67). Currently LBNP devices with relatively few restrictions on body movement in space are being evaluated in both the Russian and American space programs.

3. Reexpansion of the blood volume to pre-bed-rest levels has been achieved by oral administration of 9α -fluorohydrocortisone during the last 3 days of a period of prolonged bed rest, but orthostatic intolerance persisted (313, 317). Hyatt (157) administered daily doses during a 10-day bed-rest study and produced a blood volume expansion relative to control values before bed rest. The heart rate during post-bed-rest tilt nevertheless remained slightly elevated. Later rehydration experiments by Hyatt and West (159) tested the effects

of oral administration of saline at the end of a 1-wk bed-rest period. Saline alone did not restore plasma volume and orthostatic tolerance, but the combination of saline and a 4-h period of LBNP produced a transient return to pre-bed-rest levels. In a recent study after a 24-h period of head-down tilt at -5° (37), intravenous saline infusion sufficient to bring central venous pressure back to pretilt levels in the supine position failed to restore orthostatic tolerance but essentially abolished the loss of exercise capacity in the upright position. Normalization of central venous pressure required a volume corresponding to twice the blood volume loss.

4. Other countermeasures, including exposure to simulated high altitude (317) and mechanical devices designed to introduce sudden large longitudinal g forces (56), have generally been ineffective. Three recent approaches have provided intriguing but as yet unconfirmed results. Bhattacharya et al. (27) found that whole-body oscillation (half-sine function at 1 Hz for 20 min daily) prevented orthostatic intolerance after immersion. Prophylactic treatments with a β -adrenergic blocking agent (233) and a calcium antagonist (39) have also been used successfully in small groups of subjects.

Mechanisms. Postural hypotension, i.e., an abnormally low arterial pressure in the upright position, is a common clinical problem. Some aspects of the pathophysiology provide relevant background material for the analysis of the orthostatic intolerance after bed rest and related conditions. There are two principal primary mechanisms: 1) an abnormal degree of orthostatic central hypovolemia and 2) inadequate cardiovascular regulatory responses. The regulatory and hemodynamic patterns in the hypovolemic group exaggerate the normal response, i.e., increased adrenergic drive reflected by elevated plasma catecholamines, marked tachycardia, and vasoconstriction. The second group is heterogeneous and includes conditions that abolish or attenuate the cardiovascular responses to physiological redistribution of hydrostatic pressure gradients and intravascular volumes.

1. Hypovolemic hyperadrenergic postural hypotension [arterial circulatory anemia (33) and sympathotonic orthostatic hypotension (253)] is caused by a reduction in total blood volume or an abnormal volume distribution during orthostatic stress. Variations in total blood volume well within the physiological range affect orthostatic tolerance. Murray et al. (241) examined the combined effects of venesection and LBNP. Measurable orthostatic intolerance appeared after an acute blood volume loss of 500 ml. Bergenwald et al. (19) studied normal subjects during prolonged head-up tilt and found that fainters had significantly lower (-8%) total blood volume than nonfainters. The size of the central blood volume pool may also be an important determinant. Women have larger increases in heart rate and decreases in arterial pressure and stroke volume than men at a given degree of venous

pooling and also a smaller basal end-diastolic ventricular volume and a limited systolic reserve (101, 292).

Gaffney, Blomqvist, et al. (100, 102) have studied patients with mitral valve prolapse and hyperadrenergic postural hypotension. The functional characteristics of the abnormal valve probably contribute to the orthostatic intolerance by causing an abnormally large decrease in stroke volume in the upright position although these patients also have an inappropriately large increase in vasoconstrictor activity in the upright position and a significantly reduced total blood volume. The combination of a chronically vasoconstricted state, hypovolemia, and postural hypotension is also present in some patients with essential hypertension or pheochromocytoma.

Conditions that increase peripheral venous pooling are associated with orthostatic intolerance. Patients with massive venous varicosities or a congenital absence of the venous valves have postural hypotension and decreased exercise capacity in the upright position (26). They also have an increased compliance of non-varicose veins (363). The most important physiological mechanisms affecting the degree of pooling are linked to thermoregulation. Venous compliance and pooling are proportional to skin temperature. Heat significantly reduces and cold increases orthostatic tolerance (145, 147, 266, 267).

2. Normovolemic regulatory postural hypotension [postural (44), orthostatic (33), and asympathicotonic (253) hypotension] can be caused by 1) abnormal baroreceptor function, 2) peripheral or central lesions of the nervous system interrupting afferent or efferent neural pathways or causing dysfunction of the medullary cardiovascular control centers, or 3) end-organ failure to respond to autonomic stimuli. Such failure may be caused by nonneural lesions, e.g., lack of postural heart-rate response in patients with a degenerative disease of the sinus node (sick-sinus syndrome) or complete heart block. High- and low-pressure baroreceptor function is altered in many different cardiovascular disorders (2, 76, 82), but the efferent rather than the afferent limb is usually involved in patients with severe orthostatic hypotension. The response pattern is hypoadrenergic, with a lack of tachycardia and vasoconstriction. Parasympathetic involvement may also be a factor. Autonomic dysfunction with hypoadrenergic orthostatic hypotension may be idiopathic or one of several clinical manifestations of many different systemic and neurological disorders, e.g., diabetic neuropathy, tabes dorsalis, amyloid diseases, syringomyelia, traumatic lesions of the spinal cord, cerebrovascular disease, tumors, and degenerative or demyelinating diseases of the central nervous system (21, 25, 160-162, 212, 297, 298, 344).

Idiopathic orthostatic hypotension and the Shy-Drager syndrome are relatively rare forms. They are diseases of late middle age with general signs of dysfunction of the autonomic nervous system, e.g., bowel and bladder disturbances, inability to sweat, and im-

potence. The Shy-Drager syndrome (304) identifies a distinct group of patients who have orthostatic hypotension and signs of extrapyramidal tract involvement (Parkinson's disease); there are widespread but non-specific degenerative lesions of the central nervous system. Patients with the Shy-Drager syndrome or idiopathic orthostatic hypotension often have abnormal cardiovascular function also at rest supine. The functional characteristics of the heart suggest denervation. Stroke volume is low, perhaps because of a decreased contractility, and there is mild tachycardia at a relatively fixed rate. Peripheral resistance increases rest, but the postural vasoconstrictor responses are absent or greatly attenuated (161, 162). Humoral abnormalities can also be demonstrated. The catecholamine response to orthostatic stress is attenuated or absent (263, 361), and postural changes in plasma renin levels are often but not always (43, 212) subnormal, consistent with an efferent autonomic block. Some patients also have diminished aldosterone responses and a decreased ability to conserve sodium (142).

The hypovolemic-hyperadrenergic and the normovolemic-hypoadrenergic varieties of postural hypotension cannot always be clearly distinguished. Ibrahim et al. (160-162) have shown that patients with idiopathic orthostatic hypotension have a reduced blood volume and vasoconstriction at rest supine with increased arterial pressure and total peripheral resistance.

Orthostatic tolerance is often increased in certain clinical conditions, e.g., hypertension and congestive heart failure (3, 243, 298). Cardiac performance characteristics with attenuated preload effects on the stroke volume, decreased venous compliance, and increased total and central blood volumes together counteract autonomic dysfunction with blunted reflex responses (76, 79, 82).

Orthostatic intolerance after prolonged bed rest and related conditions. A critical analysis of the descriptive material that has been presented on the effects of prolonged bed rest and related conditions and on countermeasures identifies the following salient points:

1. Prolonged bed rest (horizontal, with head-down tilt), water immersion, and exposure to zero gravity produce actual and functional hypovolemia.

2. Postintervention cardiovascular dysfunction is characterized by orthostatic intolerance and decreased exercise capacity. Left ventricular end-diastolic volume and stroke volume are reduced in both the upright and supine positions. Intrinsic myocardial function is not depressed.

3. The degree of cardiovascular dysfunction is more severe than expected from the magnitude of the blood volume loss. Volume for volume replacement significantly improves hemodynamics but does not fully restore normal function.

4. The altered distribution of body fluids with a

headward shift is probably more important than inactivity in the development of cardiovascular dysfunction. Intermittent redistribution of venous volume (LBNP, short periods of standing) to match the conditions that normally prevail in the upright position protects against orthostatic intolerance. Vigorous exercise is less effective. Furthermore the cardiovascular dysfunction induced by prolonged bed rest or space flight is similar to that induced by periods of water immersion or head-down tilt of 24 h or less. Adaptation to inactivity, as reflected by functional and structural changes in the musculoskeletal system and the myocardium, is slower.

The discrepancy between the magnitude of the blood volume loss and the severity of the cardiovascular dysfunction suggests that adaptation produces changes in venous function, e.g., increased compliance, an abnormal pattern of postural redistribution of blood volume, systemic regulatory abnormalities, or a combination of these features. The increased heart-rate response to orthostatic stress after exposure to hypogravic conditions is consistent with a hypovolemic-hyperadrenergic pattern. However, there is only inconclusive evidence for a postadaptive increase in venous compliance and the degree of venous pooling needed to amplify the effects of the modest decrease in blood volume. Immersion produces an increase in peripheral venous compliance that persists for the duration of the intervention (81). Stevens et al. (316) found an increased venous compliance after bed rest reflected by measurements of leg volume changes during LBNP. Occlusion plethysmography during and after the Skylab-4 flight suggested increased venous compliance, whereas measurements of leg volume changes during LBNP showed increased pooling only at zero gravity (335). On the other hand estimates of the compliance of the arm (occlusion plethysmography), the leg (leg volume changes during LBNP), and the systemic veins (relating central venous pressure to leg volume changes during LBNP) showed no changes after adaptation to head-down tilt (37). Thornton et al. (335) suggested that decreased skeletal muscle tone contributes to an increased degree of venous pooling after space flight and bed rest.

Data on autonomic function after bed rest and space flight are inconclusive. As discussed earlier in this section, the postadaptive heart-rate response to orthostatic stress is increased, which may be an appropriate compensation for a decrease in stroke volume. The normal vasoconstrictor responses also appear to be intact (37). Chobanian et al. (59) found no bed-rest-induced changes in the pressor responses to norepinephrine and angiotensin infusions. Plasma catecholamines were reduced during bed rest, but the response to tilt was unchanged. The apparent turnover rate of norepinephrine was also normal. However, Schmid et al. (294) demonstrated decreased vaso- and venoconstrictor responses to intra-arterial tyramine after a 12-day bed-rest period. The responses to nor-

epinephrine did not change. These findings are consistent with normal receptor function but impaired release of endogenous norepinephrine, perhaps caused by a decreased rate of synthesis. Furthermore Billman, Dickey, Stone, and associates (28, 73) have recently documented reduced baroreceptor sensitivity and altered responses to vasoactive drugs after long-term horizontal immobilization in rhesus monkeys.

The combined data, although incomplete and inconclusive, suggest that the orthostatic intolerance after bed rest and related conditions is a multifactorial disorder. Changes in effective venous compliance and perhaps also subtle autonomic dysfunction appear to amplify the effects of a moderate absolute hypovolemia.

Dynamic Responses

Most studies of prolonged bed rest and related conditions have been limited to static before-and-after comparisons, and little is known of the dynamic response. Nevertheless there is strong evidence that the primary stimulus for the adaptation is the shift of intravascular and interstitial fluid from the lower to the upper half of the body, particularly to the central circulation. The absolute magnitude and exact time course of the responses to bed rest, head-down tilt, immersion, and weightlessness vary for specific functions, but enough similarities have been documented to justify the use of head-down tilt as a tentative model for analysis of the general features of adaptation to hypogravic conditions.

Figure 12 is based on two experiments with 24-h head-down tilt at -5° , each including five normal young men (37, 250). The base line for all measurements was supine rest. A decrease in leg volume of

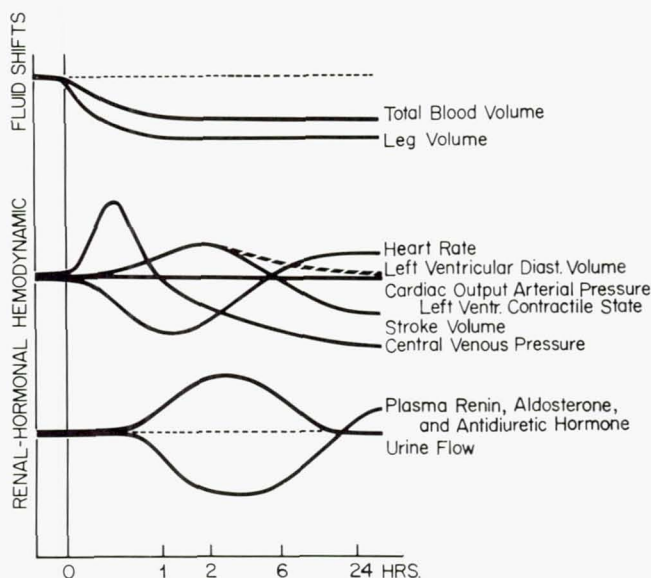


FIG. 12. Diagram of early adaptation to simulated 0 g (head-down tilt, -5°). [From Blomqvist et al. (37).]

about 1 liter during the initial 2 h indicates a central fluid shift. Central venous pressure increased significantly by 2.5 cmH₂O but returned to the base line within 1 h. This decrease was associated with a transient apparent increase in venous compliance, whereas effective systemic and peripheral venous compliances were normal at the end of 24 h. Stroke volume and left ventricular end-diastolic volume also increased transiently and reached a maximum somewhat later than central venous pressure, or at about 2 h. The contractile state did not change, nor did cardiac output. The increase in stroke volume was offset by relative bradycardia. Arterial pressures also remained unchanged. Central venous pressure and stroke volume were at 24 h below the base line, and the heart rate was slightly elevated.

Plasma renin, aldosterone, and ADH levels decreased transiently during the initial 6 h and a diuresis produced a weight loss equal to the loss in leg volume and more importantly decreased total blood volume 350 ml. Neither plasma osmolality nor electrolyte concentrations changed, but the urinary sodium/potassium excretion ratio increased.

Gauer and Henry (107) considered activation of low-pressure baroreceptors, reflex inhibition of ADH, and a water diuresis as the primary responses to a central fluid overload. The discrete venoatrial receptors are probably important (209), but impulses originating in the atrial and ventricular C fibers may also have a significant impact on renal function and the fluid-electrolyte balance (334). The role of atrial distension has been disputed (115). Epstein et al. (87, 89, 90) have clearly documented inhibition of the renin-aldosterone system and a saluresis during immersion experiments. Their data also indicate that it is unlikely that osmoreceptors or changes in renal hemodynamics have a major role. Data on fluid balance and hormonal responses at zero gravity (200) differ from the results obtained during head-down tilt and immersion. No demonstrable diuresis coincided with the early weight loss during the Skylab flights, but fluid intake decreased. Data on the early hormonal responses in space are not available. Measurements during the early recovery phase after the Skylab flight (200) and after a study on head-down tilt by Volicer et al. (343) were consistent with a relative combined inhibition of ADH and aldosterone during exposure to hypogravic conditions and a reactivation during the recovery phase. The role of other hormones (prostaglandins, natriuretic hormone) is doubtful (87).

The apparent overshoot of the hemodynamic adaptation at 24 h with a reduced filling pressure and stroke volume relative to base-line levels in the supine position supports Gauer's thesis that the upright position is the normal operating condition. When the adaptation to the hypogravic state (i.e., bed rest, head-down tilt, or space flight) is complete, hemodynamic conditions approach those that normally prevail during sitting or standing. A system adapted in this fashion

has little reserve capacity to manage additional stresses caused by postural fluid redistribution. The cardiovascular dysfunction after bed rest and space flight therefore results from a successful adaptation that has suddenly been rendered inappropriate by the reexposure to normal gravitational fields and to large hydrostatic gradients.

HYPERGRAVIC CONDITIONS

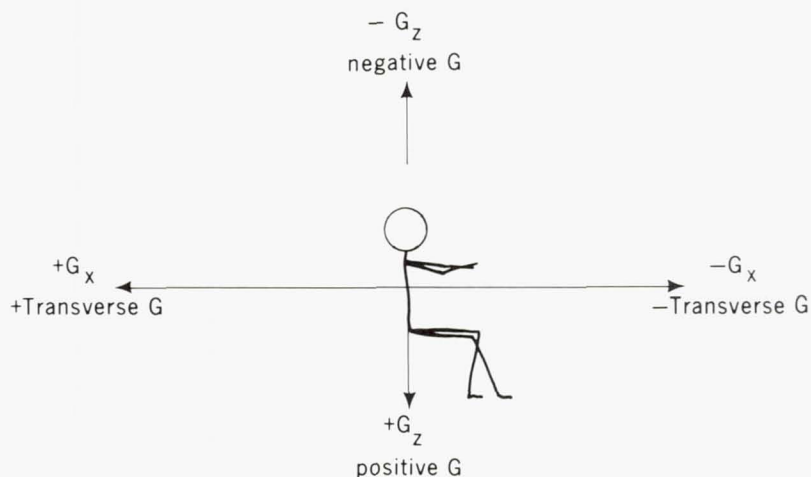
Experimental Conditions

Hypergravic conditions can be generated within the earth's gravitational field by acceleration (i.e., changing velocity or direction of motion of a body). Linear acceleration occurs when the rate of motion increases or decreases without changing direction, and angular acceleration occurs when the direction of motion changes. For example, a car that is gaining or losing speed traveling down a straight road exposes the passenger to linear acceleration, whereas going around a curve creates angular acceleration. The terms *gravitational force* and *acceleration* are interchangeable because the physical quantity of gravity is defined as an acceleration. The acceleration of a body falling freely toward the earth's surface in airless space is 1 *g*, or 981 cm/s². It is correct to speak of a gravitational force (*F*) as the product of the mass of an object (*m*) and acceleration (*a*), or $F = ma$, if the mass is a constant. Mass then becomes a proportionality factor. In biomedical applications the mass of the object being studied, either a human subject or an experimental animal, does not change significantly over the course of an experiment.

A standard nomenclature has been developed that defines the anatomical direction of the gravitational force (Fig. 13). The two gravitational forces shown in Figure 13 act either through the long axis of the body ($\pm G_z$) or across the body from back to front or front to back ($\pm G_x$). A third gravitational force, not shown in Figure 13, acts across the lateral aspect of the body ($\pm G_y$). In general the gravitational force is expressed in multiples of unity. A force of $3 + G_z$ is 3 times the normal gravitational force acting in the head-to-foot direction. In the following discussion the major emphasis is placed on gravitational forces acting in the $+G_z$ direction over periods of several seconds to minutes. Such forces may be viewed as an exaggeration of the forces acting during a change in body position from supine to upright. Gravitational forces of short duration (impact forces), i.e., 1 s or less, are not considered, and little information is available on the circulatory changes with $-G_z$ and $\pm G_y$ accelerations.

Hydrostatic pressure is a linear function of the force of gravity. Tripling the gravitational force triples the hydrostatic component of pressure along any fluid column parallel to the gravitational force. Hydrostatic pressure changes ~ 1 cmH₂O/cm vertical distance at 1

FIG. 13. Standard reference system (direction and nomenclature) for gravitational forces acting on a subject through long axis of body ($\pm G_z$), through sternum to backbone ($\pm G_x$), and through lateral aspect ($\pm G_y$, not shown).



g . At $3 +G_z$ the corresponding hydrostatic pressure change is 3 cmH₂O/cm vertical distance. In a fluid column exposed to an increased gravitational force, the local pressure at any site along the column varies directly with the position in the column either above or below the HIP.

Fluid Shifts

A $+G_z$ increase accentuates orthostatic fluid shifts from the intrathoracic compartment to the legs. Early work (104) in Germany on human volunteers demonstrated a large increase in the volume of the lower leg at 6–8 $+G_z$. Chest X-ray films taken during aircraft flight and centrifuge experiments indicated that the lung fields became clearer during increasing acceleration, which is consistent with a decrease in pulmonary blood volume or a redistribution of pulmonary blood flow. A loss of plasma volume to the interstitial space also accompanies the redistribution of blood volume. The amount of plasma volume loss depends on the duration and magnitude of the gravitational force (62, 132). Large volumes may be lost very rapidly at high G forces, e.g., 216–270 ml at 3.5–5.0 $+G_z$ maintained for 3–5 min.

The human regulatory responses to $+G_z$ acceleration have many features in common with the changes during standing. The shift of blood out of the thorax leads to an increase in plasma ADH levels (127, 183, 273). Plasma renin activity did not change with the increased gravitational force in these experiments, perhaps because of a large increase in plasma ADH or simply the short duration of the $+G_z$ exposure. Plasma renin activity increases in volunteers exposed to $+G_x$ acceleration for up to 30 min (91, 272). The increase in plasma ADH, not a result of a change in plasma osmolarity, probably is caused by the shift in blood volume and decreased activation of cardiopulmonary receptors (107, 108). In human volunteers $+G_z$ also causes a central blood volume shift that is associated with a reduction in plasma ADH and a diuresis (273). Exposure to high G forces, however, also produces

mechanical effects that may modify the regulatory responses.

Cardiac Dimensions and Performance

The heart is normally suspended by the central vasculature and the pericardium, which tends to make the heart vulnerable to increased gravitational forces, particularly to $+G_z$ acceleration. Gauer (104) initiated radiographic studies of the response to hypergravic conditions in cats and monkeys in 1936. Centrifuge experiments showed that $+G_z$ acceleration pushed the heart caudally and reduced both systolic and diastolic volumes. The apical portions of the ventricles were devoid of contrast material during systole. Contrast material was injected intravenously prior to the acceleration exposure, and no distinction could be made between the right and left sides. The obliteration of the apical cardiac chambers during $+G_z$ acceleration led to the hypothesis that the endocardial walls may actually rub against each other during systole, causing cardiac damage (111).

The end-systolic volume of the ventricles depends on the level of autonomic nervous system activity (contractile state) and the pressure in the aorta during ejection (afterload). The level of $+G_z$ acceleration greatly influences direct mechanical effects, the autonomic activity, and the aortic pressure. Erickson, Sandler, and Stone (93) studied anesthetized dogs during $+G_z$ acceleration and also demonstrated that the heart was progressively shifted caudally as the level of $+G_z$ acceleration was increased. In recent studies with similar conditions H. Sandler (unpublished observations) obtained cineangiograms from anesthetized dogs during $+G_z$ acceleration. Left ventricular diastolic and systolic volumes were reduced, but there was no evidence that the walls of the left ventricle actively hit each other during systole. The aorta and vessels leaving the arch of the aorta are stretched during $+G_z$ acceleration, and the diameter of the vessels is reduced. The coronary vessels apparently are not involved, and mechanical obstruction

during $+G_z$ acceleration is unlikely (93). During transverse acceleration ($+G_x$) the heart is displaced toward the posterior portion of the thorax with minor changes in ventricular chamber dimensions (290).

The electrical activity of the human heart during acceleration stresses has been studied extensively for more than 35 years in both centrifuge and aircraft experiments. Gauer (104) described flattening of the T waves in the limb leads, sometimes associated with S-T segment elevation at higher acceleration levels. Cardiac dysrhythmias ranging from atrioventricular block to premature ventricular contractions were also seen. Gauer noted the similarity between these changes and those during myocardial ischemia and suggested that coronary flow was compromised during acceleration stress. In a subsequent study Zuidema et al. (364) found potentially serious cardiac arrhythmias in four subjects, one of whom also had chest pain. These findings may support the view that myocardial ischemia limits the ability of humans to tolerate $+G_z$ acceleration. However, acceleration levels as high as $9 +G_z$ (50, 63, 114, 153, 300) have failed to produce any significant arrhythmias persisting after deceleration. In several studies premature ventricular contractions and multiform arrhythmias were observed during the early portion of the various acceleration profiles, but they disappeared either before or at the termination of the acceleration stress. This time course is inconsistent with ischemia. The movement of the heart in the chest cavity and increased efferent cardiac sympathetic activity could explain the electrocardiographic (ECG) changes in normal volunteer subjects. β -Adrenergic blockade prevents the T-wave changes induced by acceleration (63).

Blood flow to the myocardium during $+G_z$ acceleration has been examined in experimental animals (58, 93, 198, 300, 320), but the results are inconclusive. Chimoskey (58) found a decrease in coronary flow when unanesthetized dogs were exposed to brief durations (10 s) of $+G_z$ acceleration. The decrease in coronary flow coincided with the development of T-wave changes of the ECG. Shubrooks (300) found that coronary flow increased up to $3.5 +G_z$ in anesthetized dogs during the early portion of the acceleration stress and either remained elevated or returned to control levels by the end of the 60-s stress period, whereas Erickson, Sandler, and Stone (93) found a decreased coronary flow in anesthetized dogs exposed to up to $4 +G_z$. As coronary flow decreased so did coronary sinus oxygen saturation. This study would suggest some potential for myocardial ischemia. Using unanesthetized miniature swine, Stone et al. (320) found that coronary flow decreased up to levels of $9 +G_z$ with the most significant decreases above $5 +G_z$. Using microspheres to measure coronary flow and distribution in miniature swine, Laughlin et al. (198) found that coronary flow increased up to $7 +G_z$. They used aortic pressure as a reference point for the measurement of coronary flow, however, and made no measurements

if the pressure fell below 100 mmHg. This and other variations in methodology may at least partially explain the divergent results of the flow measurements.

Flow measurements cannot provide information on the potential for ischemia without data relating to myocardial oxygen demand. The effect of $+G_z$ acceleration on the oxygen demand of the heart and on the balance between oxygen demand and supply is uncertain. Demand probably increases because of the increase in heart rate with maintenance or elevation of aortic root pressure, but the effect on wall tension is modified by a changing cardiac chamber geometry and probably also by changes in the contractile state.

Ultrastructural and biochemical changes have been observed in hearts taken from miniature swine immediately after exposure to various levels of $+G_z$ acceleration. Burton and MacKenzie (52) found left ventricular subendocardial hemorrhage that was directly related to the level and duration of acceleration exposure. Heart rate and/or the sympathetic nervous system were involved because β -adrenergic blockade reduced heart rate during acceleration and reduced the degree of subendocardial hemorrhage. In the same animals MacKenzie et al. (215) studied the ultrastructural changes and found myofibrillar degeneration, pooling of mitochondria, and involvement of the Purkinje fibers. Lindsey, Stone, et al. (210) also examined the ultrastructural changes of myocytes from swine exposed to $9 +G_z$. They found changes similar to those described by MacKenzie et al. (215) and also evidence of disruption of contractile fibers in some cells. Biochemical studies (78) have demonstrated an increase in proteolytic enzyme activity in the myocardial cells up to 2 wk after acceleration exposure. Whether these signs of myocardial damage are caused by ischemia, hypoxia, or increased release of catecholamines is not known. How significant these findings are to potential hazards of acceleration stress ($+G_z$) to humans is uncertain, but they should not be ignored.

Cardiac Output and Regional Flow

The ability of the cardiovascular system to withstand $+G_z$ acceleration is defined clinically by the appearance of limiting symptoms. The usual endpoint criteria for measuring acceleration tolerance progress from the loss of vision to unconsciousness. Loss of vision usually occurs around $4.0 +G_z$ and blackout occurs around $4.5 +G_z$ in relaxed humans (153). These endpoints vary considerably between and within individuals, and the threshold level can be altered by protective devices (anti-G suit) or maneuvers (straining). Lambert and Wood (193) measured eye-level arterial pressure in volunteers and found that vision was lost at an average arterial pressure of 20 mmHg. Figure 14 is a representative graph of the acceleration tolerance for $+G_z$. Levels of acceleration and times of exposure below the curve can be well tolerated by most relaxed humans.

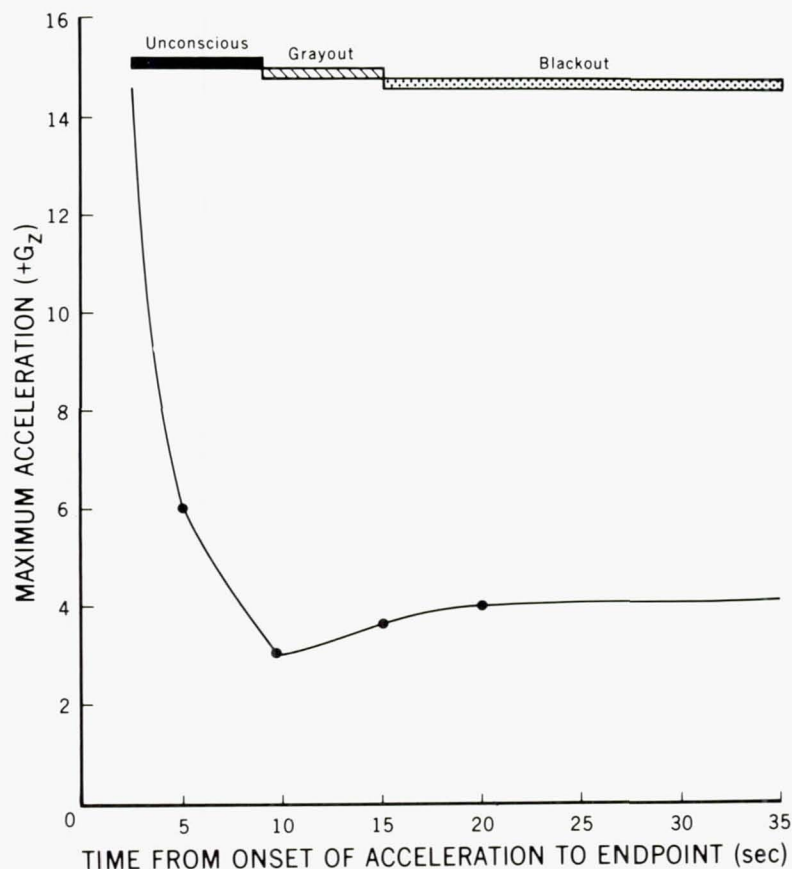


FIG. 14. A representative average curve for $+G_z$ acceleration tolerance in relaxed humans in seated position. Top of graph, reported symptoms at these acceleration levels. Increased tolerance above 10 s is probably circulatory reflex compensating for increased gravitational force.

Shubrooks (300) found that heart rate increased in an almost linear fashion with increasing $+G_z$ acceleration up to levels of $\sim 6 +G_z$ in subjects protected from the usual endpoints of acceleration stress. Maximal heart-rate responses were achieved within 3–12 s after the occurrence of peak G levels, and rates tended to remain at this level for the duration of the experiment. Peak heart rates measured in this study ranged from 155 to 205 beats/min and were not related to the preacceleration heart-rate level. When the acceleration is terminated a considerable bradycardia usually ensues. The increase in heart rate in human subjects during $+G_z$ acceleration appears to be mediated mainly by the sympathetic nervous system with a minor contribution from vagal withdrawal (34, 35). The relative importance of sympathetic and parasympathetic mechanisms, however, is likely to vary with preexisting or background levels of autonomic activity (270). Upon cessation of the acceleration stress atropine can block the bradycardia (205), which clearly indicates a strong reversal of the autonomic nervous system balance occurring at the sinoatrial node. During $+G_x$ acceleration the heart rate also increases but less than during $+G_z$ acceleration. During $-G_z$ acceleration the heart rate decreases as the pressure rises in the region of the head and the carotid sinus.

Cardiac output and stroke volume have been measured in human volunteers during $+G_z$ acceleration

(207, 208, 275, 357). Table 3 shows representative results. Both cardiac output and stroke volume fall progressively with increasing gravitational force, but the aortic pressure measured at the heart level actually increases, indicating peripheral vascular adjustments during this type of stress. The decrease in cardiac output is the result of the peripheral shift in blood volume, a decreased venous return, and a reduced end-diastolic volume of the ventricles. Rosenhamer (275) found that cardiac output at $3 +G_z$ remains about 3 liters/min below the expected levels at $1 +G_z$ also during exercise. However, the rate of increase in cardiac output relative to oxygen uptake is similar at 1 and $3 +G_z$, or about 6 liters/min for a 1 liter/min increase in O_2 uptake. Stroke volume also remains subnormal during exercise at $3 +G_z$, but the increase on transition from rest to exercise is larger than at $1 +G_z$.

Cardiac output in the human does not decrease during $+G_x$ acceleration (Table 3). Results were similar during moderate levels of $+G_x$ acceleration in experimental animals. Arterial pressure referenced to heart level was maintained and even increased slightly. There was a small decrease in stroke volume. A decrease in cardiac output was recorded at $15 +G_x$ (318). Exceedingly high levels of $+G_x$ acceleration probably compromise ventricular filling when the heart is being pushed against the posterior chest wall.

PULMONARY BLOOD FLOW. The effect of $1 + G_z$ on the regional distribution of pulmonary blood flow is well documented, and an increase in $+G_z$ acceleration is expected to accentuate it (352). Blood flow in the lungs shifts from the superior region of the lungs (apex) toward the more dependent lung region (base). This might cause greater right-to-left shunting of blood, which would reduce the oxygen content of arterial blood. Decreases in arterial oxygen saturation have been demonstrated in both humans and experimental animals exposed to acceleration stress (13–15, 252, 268, 312, 356). The decline in arterial oxygen content is progressive during $+G_z$ acceleration and can lead to saturation values less than 80% at $5 + G_z$ (356). Oxygen breathing reduces but does not abolish the decline in oxygen saturation (14). The effective alveolar-arterial oxygen difference decreases on the transition from rest to exercise at normal gravity but increases during exercise at $3 + G_z$, presumably because of an increase in the number of alveoli with low ventilation/perfusion ratios (275). During $+G_x$ acceleration arterial oxygen saturation at rest can decline to as low as 75% at $8 + G_x$ (312). The increase in accelerative forces increases alveolar size in superior lung regions and decreases it in the dependent regions, causing a greater ventilation/perfusion mismatch (57, 131, 339).

BRAIN. The distribution of cardiac output has been examined to a limited extent in human volunteers and in experimental animals. As mentioned previously, loss of vision and unconsciousness are experimental endpoints for the termination of $+G_z$ acceleration. Clearly blood flow to the head must be reduced, since arterial pressure at eye level can fall below the range of cerebral blood flow autoregulation (149, 193). Total perfusion of the brain measured by the radioisotope-clearance technique was reduced at 2, 3, and 4 $+G_z$ in humans. The long time required to accomplish these measurements precluded studies at higher levels of acceleration (155) and also raises some questions concerning the measurements at 2–4 $+G_z$, since a steady-state condition is a prerequisite for accurate measurement. In anesthetized primates, cerebral blood flow remained almost constant until arterial pressure at the base of the brain dropped below 65 mmHg at which point cerebral flow declined as arterial pressure fell (H. L. Stone, unpublished observations). This occurred at 3.0–3.5 $+G_z$ in all animals studied. Clearly there is no way to relate this level to loss of vision in these anesthetized animals, but the decreasing pressures strongly suggest a reduction in blood flow to the brain. Cerebral blood flow has not been measured during $-G_z$ acceleration, but there is no reason to suspect that it is compromised.

SPLANCHNIC AND MUSCLE BLOOD FLOW. Howard and Garrow (154) and Stone (318) measured forearm blood flow, splanchnic blood flow, and arterial pressure in six normal volunteers (Fig. 15). Forearm blood flow, measured by a mercury-in-Silastic strain gauge, de-

creased considerably at $3 + G_z$ compared to 1 g. Resistance increased, indicating active vasoconstriction. Splanchnic blood flow (indocyanine-green clearance) changed little, and the pressure-flow ratio increased, suggesting splanchnic vasoconstriction. In experimental animals (322) splanchnic blood flow decreased drastically during acceleration when the position of the animal's body was changed from a $+G_x$ acceleration position to a $+G_z$ acceleration position. Greenleaf et al. (127) measured leg blood flow in three subjects exposed to increasing levels of $+G_z$ acceleration. Flows decreased from mean resting values of $6\text{--}9 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ ml}^{-1} \text{ tissue}$ to $3\text{--}4 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ ml}^{-1} \text{ tissue}$.

Tissue flows, when measured during $+G_x$ acceleration, change little up to about $12 + G_x$. Above this level, tissue compression may contribute to flow reduction. Peripheral vascular damage can occur when the intravascular pressure (or force) exceeds the tensile strength of the surrounding vascular wall. Small cutaneous blood vessels are ruptured in humans exposed to high levels of $+G_z$ acceleration, particularly around the ankles and in areas of the legs not protected by a pressure suit. The areas immediately under the pressure garment are exposed to a static counterpressure that prevents the vessels from rupturing. It is not known if human deep muscle vessels also rupture, but it does not seem likely because of the muscle pressure usually generated to sustain the high levels of $+G_z$.

In summary, with increasing $+G_z$ acceleration, cardiac output falls, stroke volume falls, heart rate increases, arterial pressure at eye level falls while the arterial pressure at the aortic arch increases slightly, cerebral blood flow falls, and the peripheral blood flow decreases. Arterial oxygen saturation decreases because of an increased shunting of blood through the lungs.

Dynamic Responses and Reflex Adjustments

The hemodynamic responses to $+G_z$ acceleration are similar to those during orthostatic stress and hemorrhage, including a reduction in arterial pressure in the head region. Activation of the arterial baroreceptor reflex explains the increase in heart rate and the peripheral vasoconstriction. In 1933 Jongbloed et al. (175) sectioned the carotid sinus nerve and abolished the tachycardia response to $+G_z$ acceleration in experimental animals. Greenfield (125) found that the compensatory rise in arterial pressure during prolonged acceleration did not occur after stripping the carotid sinus region.

Other reflexogenic areas may be involved to either amplify the carotid sinus reflex or attenuate the response (32). Attenuation of the baroreceptor response may occur through activation of vagal cardiopulmonary mechanoreceptors and prevent the maximum compensatory effect. Plasma levels of ADH increase during $+G_z$ acceleration (127), which is consistent with activation of cardiopulmonary receptors. Other recep-

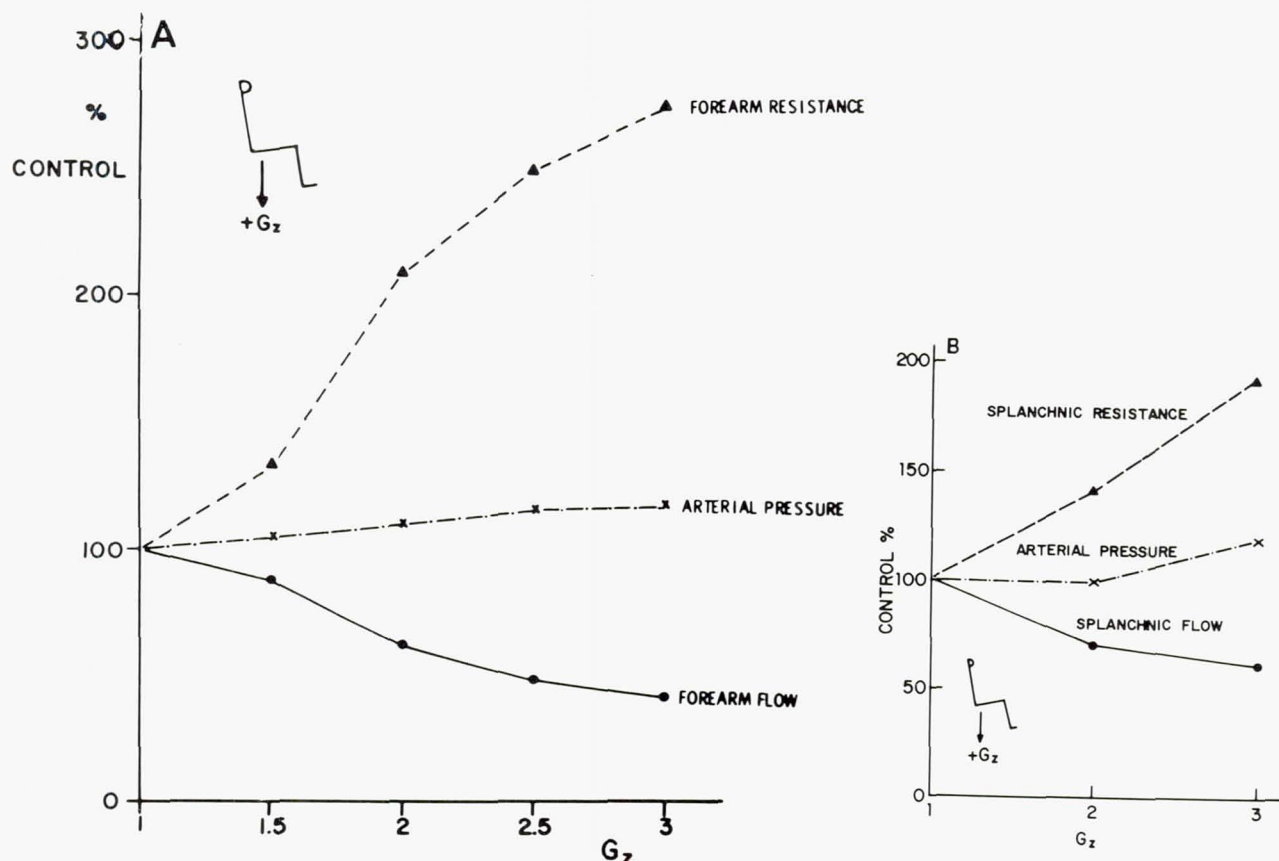


FIG. 15. Average % change from control in forearm blood flow and resistance (A) and splanchnic flow and resistance (B) in 6 volunteers. Arm suspended at heart level throughout experiments. Arterial pressure was referenced to heart level. [Adapted from Stone et al. (318) and H. L. Stone, unpublished observations.]

tor areas, such as the splanchnic and skeletal muscle regions, could also send afferent information to the central nervous system to modify the overall responses to this type of stress. This area has not been investigated extensively and needs future work.

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Bones and Stones in Space—Integrating the Medical and Scientific Questions

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ABSTRACT

Bone loss and muscle atrophy are two consequences of long-term spaceflight, and neither the underlying mechanisms nor effective countermeasures have yet been found. Experiments designed for space station and beyond incorporate a number of scientific objectives focused on two interrelated concepts, calcium homeostasis and bone homeostasis. The experiments to be done require a trade-off between ground based analysis of samples and development of instruments to do these studies inflight. The scientific community is currently in the process of defining which scientific objectives can be accomplished inflight and what instrumentation is required to do this.

WITH THE COMING OF THE SPACE STATION and other possible scenarios for long-term exposure of man to microgravity or reduced gravity, the major emphasis in biomedical research and development has shifted away from solution of the short-term consequences of microgravity such as space adaptation syndrome to two major concerns, musculoskeletal deconditioning and radiation exposure. The short-term effects still exist, and will be a continuing concern for shuttle flights, especially during station construction. However, in planning for long-term habitability our major concerns are 1) to provide for the health and safety of the crew in flight, 2) to maintain the capability

of the crew to work efficiently under conditions of reduced gravity, and 3) to avoid any medical consequences on return to earth associated with long-term exposure to reduced gravity. NASA and the biomedical research community have long recognized that these objectives can only be met through an integrated research program designed first to understand the basic mechanisms underlying the biomedical problems and then to design countermeasures to prevent these problems from becoming risks. The realities of manned spaceflight unfortunately dictate a different approach, because in many cases we need humans either to do experiments with model systems or to act as experimental subjects to get at these basic questions. Therefore, we must plan our program in a non-risk-free environment, and to do this we must anticipate the unexpected, plan for it, and hope for the best.

PHYSIOLOGY OF BONE LOSS

Musculoskeletal deconditioning during exposure to reduced gravity has many facets, and we must consider them all in our approach to this problem. Figure 1 shows a model of the interrelationships among the four major components in the calcium/bone system: blood and extracellular fluid (ECF), intestine, kidney, and bone. (Figure 1) Under normal circumstances, blood calcium is regulated very tightly at about 10 milligrams per 100 ml of serum, both in man and in other animal species. Deviations of as little as 0.2 mg/100 ml, or 2%, are enough to invoke

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feedback mechanisms; release of parathyroid hormone (PTH) when blood calcium drops and release

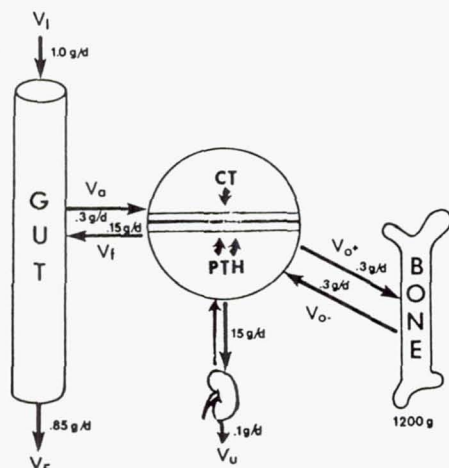


Figure 1. Representation of the relationships among various components in the calcium/bone homeostatic systems. Transport of calcium into and out of the compartments is shown in terms of grams/day.

of calcitonin when it gets too high. In actuality, it is not the total blood calcium invoking this response, but the fraction of it, about 40%, that is not bound to albumin and other proteins, that is, the ionized calcium fraction. This fraction is sensitive to many factors, including blood pH (acidity) which can be affected by CO₂ in the air, protein content of the blood, and the fraction of red cells to plasma (hematocrit). The tight regulation of blood ionized and total calcium is termed "calcium homeostasis".

The blood and ECF calcium pool is about five grams of calcium in a normal human. In our diet, we consume about one gram of calcium daily and 300 milligrams of this gets absorbed into the blood with the rest passing through the intestine. The digestive juices secrete about 150 mg of calcium back into the intestine, so net absorption is only 150 mg. Under normal circumstances, the kidney filters about 15 grams of calcium per day and reabsorbs 99%, so that only 150 mg is excreted in the urine. Bone is a dynamic organ, constantly being reabsorbed and rebuilt, at a rate of about 300 mg

per day averaged over the whole skeleton, or about 10% of total skeletal mineral per year. Some bones turn over more rapidly, such as 25% per year for the spine, and some more slowly like the femur at 1-2% per year. Bone is also a reservoir for calcium homeostasis in pathologic conditions, so that blood calcium can be maintained at the expense of the skeleton.

In the past, most studies of calcium metabolism were focused on the calcium homeostatic system described above. Recently, researchers have recognized that two interrelated systems are actually present, a calcium homeostatic system and a bone homeostatic system. This concept is extremely important, because now we can see that the skeleton is not simply a reservoir to maintain calcium homeostasis, but can actually act as an input to drive it when the skeleton responds to an external stimulus. In looking at Figure 1, we see that there are two major control points in the maintenance of skeletal integrity and calcium homeostasis. The first is the kidney. If something happens to reduce tubular reabsorption of calcium, even from 99% to 97%, urinary calcium output would triple, blood calcium would drop, and skeletal calcium would be mobilized to maintain blood calcium. The other input to blood calcium, the gut, would not begin to compensate for such a major calcium loss, so the skeleton would lose mineral over the long term. Even so, with a tripling of urine calcium we are only talking about 1% per month bone loss.

The second control point is the skeleton itself. An external stimulus with an apparent direct effect on bone, for example a major reduction in blood estrogen levels in women, can change the "set point" for maintenance of skeletal integrity, that is, the body decides it does not need the amount of bone that it has. The breakdown of the skeleton increases, dumping calcium into the blood. The calcium homeostatic system is overwhelmed and increases excretion and decreases absorption of calcium in an attempt to maintain serum calcium. After some time, the new "set point" is approached and the feedback systems return to normal. Figure 2 demonstrates this concept in

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postmenopausal women. Immobilization or microgravity apparently has a similar effect. (Figure 2.)

When normal mechanical stresses of weightbearing and muscle pulls are removed from the skeleton, breakdown of bone increases and formation

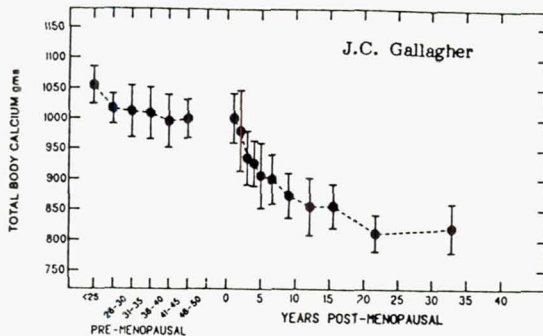


Figure 2. Total body calcium in normal, untreated women. After menopause, an exponential decline in bone mass occurs, reaching a new plateau about 8-12 years post-menopause, at a value of 80-85% of initial. This suggests that the decline in reproductive hormones (estrogen, progesterone) represent a reduction of 15-20% in the body's "need" to maintain the skeleton at its premenopausal level.

decreases. This has been well documented in immobilized and paralyzed patients (1, 2)* and in animals in spaceflight (3, 4). As with estrogen deficiency this process appears to be self-limiting (5, Figure 3).

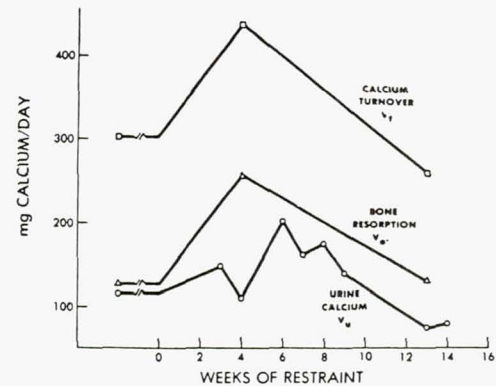


Figure 3. Parameters of calcium metabolism in an immobilized monkey show an acute increase of bone to rapid bone loss, with a later resorption and urine calcium, leading return to below-normal levels after the skeleton has reached its new equilibrium.

In paralyzed adult humans, about 40-50% of total skeletal mineral is eventually lost if no treatment is given, so it appears that the "set point" for the skeleton has been reduced by this amount. We can raise this set point back to normal by restoring normal weightbearing for at least part of the time (6), or we can raise it above normal by increasing mechanical stresses on the bone (7, 8).

The design of both our basic experiments to understand how and why bone is lost in microgravity and our countermeasures to prevent this loss are underpinned by these two concepts of calcium homeostasis and bone homeostasis. The interrelationships between blood calcium, increased excretion of calcium by the kidney and risk of formation of kidney stones, the stresses exerted on bone by skeletal muscles or weightbearing-like forces, handling of calcium by the intestine, and interaction of pharmaceutical agents must be understood before we can say that bone and muscle loss is no longer a barrier to long-term habitation at reduced gravity. However, our experiments have just started, and we must now think in terms of designing new experiments with tools to solve this problem.

INSTRUMENTATION

* References indicated in parentheses.

The instrumentation we need to do biomedical and basic biological research in the musculoskeletal area is varied, and includes both inflight tools as well as ground-based systems. We can categorize the instrumentation in several ways, and I will discuss one approach based on our research questions, the model systems we use, and the types of experiments we must do both for the study of calcium homeostasis and bone homeostasis.

HABITATION - The first instrument we need for any research is a habitat for the research subjects, and that habitat will have certain requirements for both liveability and experimental procedures. For studies of calcium and bone homeostasis we have three primary animal species we use: humans, rhesus monkeys and rats. We are studying the effects of reduced gravity on these species and our long-term goal is to do experiments at zero-g, one-sixth-g, one-third-g and one-g in space, based on long-term agency objectives for man in space, lunar exploration and Mars exploration. This requires a variable gravity habitat for all three species. At the present time, perhaps the most cost-effective means of doing our research is to build a tethered system with a habitat module at one end for humans and a habitat/laboratory module at the other end for animal studies. This configuration would give us the option of zero-g (non-rotating) or up to 1 or 1.2 g with rotation about the center of the tether, would provide bioisolation for living areas, and would allow us to put sophisticated laboratory equipment in the animal module which could double for analysis of human samples brought over from the human module. In addition, because we would have isolated the animal module completely we can more easily use two radioisotopes we require for much of our work, carbon-14 and tritium, without worrying about C-14-carbon dioxide in the air or tritiated water in the condensate recovery system for the human module. We can also control the atmosphere in the animal/lab environment to do such experiments as the effect of atmospheric CO₂ on blood chemistry in microgravity. The requirement to

make a man-rated habitat at 1 g would define the length of the tether, and we would have no problem with coriolis forces in our monkey or rat studies.

Included in the design of the habitat must be a means for continuous and/or periodic waste collection for metabolic studies in all species. In rats, while urine collection and separation from feces is desirable, it is difficult even in our ground-based studies because urine volume is so low. This is not a major concern, however, because calcium output in rat urine is also quite low, and combined urine/fecal collections have been used quite successfully in flight experiments (9). For rhesus monkeys, long-term (90-120 days) habitation is a more difficult problem. First, a cage must be designed which allows complete monitoring of food and water intake, collection of waste, separation of urine from feces at least some of the time, and access to the animal for health purposes while maintaining bioisolation. A waste management system that works must also be designed for the human habitat.) An additional requirement for experimental purposes with the monkey is the capability to remove the monkey from the habitat into an experimental module such as the RHEC, developed by the French as part of the ESOP facility for large primates to be flown on Spacelab. This will allow hands-on provocative testing to be done with the animal restrained for short periods of time during a long-duration mission.

GENERAL LABORATORY EQUIPMENT - Many of the studies proposed for space station and beyond in the bone/calcium discipline require general laboratory equipment already developed or under development for Skylab or Shuttle/Spacelab. This includes such equipment as a blood drawing kit, refrigerated centrifuge, 4 C refrigerators, -20 C and -70 C freezers, work station with microscope and small animal surgery area, and the like. However, there are three approaches to the development and use of more experiments-specific but still generally useful equipment. First, we can define those measurements which must be made in flight either non-invasively or because samples

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obtained must be fresh and cannot be fixed or frozen. This includes such things as blood gases and ionized calcium measurements and measurements made non-invasively for which the time course is the highest scientific priority such as regional bone and muscle density and volume or tissue perfusion. Equipment to make these measurements must be flight qualified. Other samples can be obtained, minimally processed, frozen or fixed, and returned to ground-based laboratories for analysis, or pre and postflight non-invasive measurements can be made on experimental subjects. This first option requires, in addition to some equipment development, a significant amount of stowage and sample return capability. It has the advantage that when a sample is returned to an investigator's laboratory the same high quality, high precision measurements made on routine specimens can be made on these samples, and also has the advantage that other analyses not planned for originally but of significant scientific interest can be done. The disadvantage is that measurements cannot be made during the course of the experiment, the data interpreted, and the experiment modified if necessary to optimize the scientific output. For human studies, it also has the disadvantage that clinically-relevant changes occurring in individuals may not be apparent until postflight measurements are made.

The second option is the development of most or all of the instrumentation needed to make measurements in flight. The main advantage of this option is that it substantially reduces stowage and sample return requirements. Additionally it allows data to be transferred to the investigators during the course of an experiment, allowing them to modify the experimental protocol in near real time. The disadvantages of this approach module 1) loss of precision of measurements compared to an investigator's laboratory, 2) the need to train astronauts in many more laboratory techniques than is currently done, 3) increased crew time requirements, and 4) long lead time to develop sophisticated automated analysis and testing instruments, many of which do not

currently exist even in the laboratory.

The third option is a mix of the first two, with a balance between their advantages and disadvantages. this would include development and flight qualification of instrumentation that the scientific community feels can be used to make measurements with sufficient accuracy and precision to satisfy their scientific requirements. For example, the simple serum chemistry analyses developed for the Health Maintenance Facility lab will not begin to suffice for research purposes, but flight qualification of a routine blood gas analyzer will do. Of particular interest will be capabilities to measure minerals and other materials in excreta for metabolic studies, because this will comprise a large fraction of the stowage requirements for the bone/calcium discipline.

EXPERIMENT-SPECIFIC INSTRUMENTATION-Some scientific objectives in the bone/calcium discipline require specific instrumentation. High precision, high accuracy instruments have been developed for NASA for pre and postflight measurements of bone and muscle density and volume. Figure 4 shows a schematic of part of the Computed Tomodensitometer (CTD), a computed tomography scanner designed and built for delivery to Johnson Space Center by the University of California, San Francisco. This device will measure bone density in any region of the body, in three dimensions, with an accuracy and precision of better than 0.5%. It can also do routine CT cross-sectional imaging to detect kidney stones, for example, and can be used for tissue perfusion measurements (using inhaled stable Xenon gas), cardiac dimensions using ECG-gated CT, or other studies requiring three-dimensional imaging/density capabilities. The prototype device is a ground-based model, but the system design is inherently low power, low mass and low volume, so it can be optimized for a flight instrument. Some of the highest priority science objectives in the bone/calcium discipline, especially for human subject, require determination of the time course of regional changes in bone density,

both the natural course and the changes that may occur in response to specific exercise regimens used as

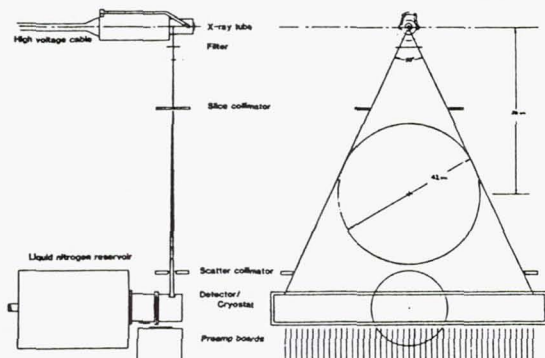


Figure 4. Diagram of the source/detector system of the UCSF/NASA Computed Tomodensitometer. The source is a low-power, heavily stabilized and filtered x-ray tube producing a dual-energy photon spectrum simulating a radioisotope such as gadolinium-153, but with 50-100 times the output. the detector is high-purity germanium, cooled either with liquid nitrogen or a small refrigerator with a capacity of a few watts. the custom data acquisition electronics count photons at 1 MHz per channel, 2 channels per detector, 360 detectors, and dump data to a VME-compatible system for processing.

possible countermeasures. this is especially important in light of recent evidence that even 2-3 hours per day of exercise may not be sufficient to protect against muscle atrophy in flight (10).

Several other instruments will have to be developed and flight-qualified to investigate specific problems in the musculoskeletal area. Non-invasive measures of bone quality or mechanical strength will be necessary in serial studies in humans and monkeys. Some use of radioisotopes will be required in experiments in rats and monkeys, necessitating proper handling procedures. Long half-life isotopes may be used, but some studies may require the use of on-board counting facilities. Alternatively, stable isotopes may be used as tracers, with return of samples for analysis processing bone specimens from rats or monkeys will be necessary,

including such items as vacuum ovens, microtomes autoradiographic materials, and microscopes with video interface. It may be of interest to eventually develop a nuclear magnetic resonance (NMR) spectrometer to do in vitro or in vivo small animal metabolism studies of phosphorus, carbon-13 and hydrogen-containing compounds. In general, most of the instrumentation we need for specific experiments exists in the laboratory. It just remains to 1) decide whether we need it in flight, based on our choice of the options presented earlier, and 2) develop flight-qualified versions that can perform up to the standards we set for obtaining high-quality scientific results.

SUMMARY

Musculoskeletal research for space station and beyond is high priority because of the adverse long-term consequences to crew health and performance due to muscle and bone atrophy. Research is driven by two concepts, calcium homeostasis, or maintenance of normal blood calcium levels, and bone homeostasis, or the response of the skeleton to its environment. Scientific objectives require the use of multiple species to do experiments, including humans, rhesus monkeys and rats, and the use of a variable gravity habitat for all three species. Much of the general laboratory instrumentation needed for experiments has been developed previously, but specific items remain to be optimized and flight qualified. Most equipment for specific experimental objectives exists in ground-based laboratories, but for high-priority studies this equipment must be further developed flight-qualified.

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Cell Sensitivity to Gravity

Abstract. Cultures of human lymphocytes exposed in microgravity to the mitogen concanavalin A showed less than 3 percent of the activation of ground controls. This result supports the hypothesis, based on simulations at low g and experiments at high g, that microgravity depresses whereas high gravity enhances cell proliferation rates. The effects of gravity are particularly strong in cells undergoing differentiation.

An answer to the question of whether cells are sensitive to gravity was one of the objectives of experiment IES031, on the effect of weightlessness on lymphocyte proliferation, performed on board Spacelab 1. Another objective was to establish, by exposing cultures of human lymphocytes to a mitogen during space-flight, whether functional changes occurred in the cells responsible for the immune response. Several investigators (1) reported that lymphocytes from the majority of crew members on U.S. and Soviet space missions had a decreased response to mitogen after flight. Stress may be one of the reasons for the reduction of lymphocyte response (2). A manifold increase of induced interferon pro-

duction was recently observed in cultures of human lymphocytes flown on the Soviet space station Salyut 6 (3). One of the few experiments dedicated so far (4) to the study of the effects of microgravity on cells was performed on Skylab with a strain of human embryonic lung cells, WI-38 (5). Although there were no significant changes in cell proliferation and motility, glucose consumption was significantly lower in the cells flown in space.

On Spacelab 1 we exposed cultures of human lymphocytes to concanavalin A (Con A), a mitogen capable of transforming resting T lymphocytes into activated (dividing) cells. This is a suitable model for the study of certain aspects of the immune system and of cell differentiation in vitro. In addition, in connection with the experiment on Spacelab, we studied the adaptation of animal cells to an altered gravitational environment and found that proliferation of five types of cells is remarkably enhanced at 10 g (6), whereas glucose consumption is the same at both high g and 1 g. However, cell motility is hindered at 10 g. In particular, lymphocyte activation is strongly gravity-dependent; the proliferation rate is almost doubled at 10 g. We suggest that, under gravitational stress, the cell is capable of switching to other metabolic pathways (6). Conversely, when lymphocytes are cultivated at simulated microgravity in a rapidly rotating clinostat, in which gravity is changed from a vector to a scalar in the presence of Con A, activation is depressed by 50 percent (7). In general, hypergravity promotes cell proliferation, whereas microgravity has a depressing effect. Our experiment on Spacelab 1 was designed to test this hypothesis. All preflight operations and the synchronous ground control experiment were performed at Kennedy Space

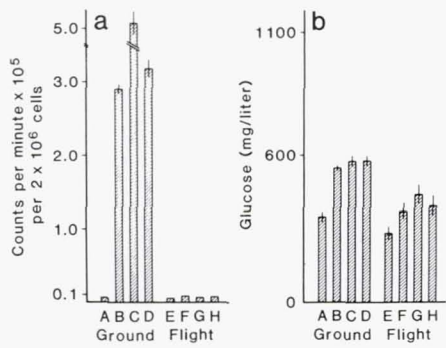


Fig. 1. Lymphocyte activation induced by Con A in microgravity. Cultures of human lymphocytes were exposed to mitogenic concentrations of Con A in ground samples B, C, and D and flight samples F, G, and H, respectively. Samples A (ground) and E (flight) were unstimulated controls. (a) Activation measured after 69 hours incubation at 37°C as [³H]thymidine incorporation into trichloroacetic acid-precipitable material. (b) Glucose remaining in the medium measured by the glucose dehydrogenase method (6). The initial concentration of glucose in the medium was 1100 mg/liter. The standard deviation of triplicate samples is given, except for samples A, E, F, G, and H in (a), for which it was too low to be shown here.

Center. The analysis of the flown and control samples was performed in Zürich.

The apparatus (4) consisted of a carry-on incubator operating at 37°C, containing four cell culture flasks, syringes with mitogen, ^3H -labeled thymidine, and hydroxyethyl starch (HES) as a cryopreservative. Twelve hours before launch, lymphocytes were purified from human blood and resuspended in culture medium (6) at a final concentration of 2×10^6 cells per milliliter. Portions (8 ml) of the culture were sealed in eight flasks: A, B, C, and D were the ground control samples and E, F, G, and H the flight samples. The flight incubator was carried on board 6 hours before launch; 6 hours after liftoff the incubator was installed and switched on. The experiment was activated by injection of Con A (25 $\mu\text{g}/\text{ml}$) into three flight and three ground cultures. The fourth ground (A) and flight (E) cultures were unstimulated controls. After 69 hours of incubation, radiolabeled thymidine was injected into cultures to give 4 $\mu\text{Ci}/\text{ml}$. Two hours later HES was added to a final concentration of 14 percent. Air was let into the flasks and, after vigorous shaking, the cell cultures were stored in liquid-nitrogen freezers both on board and on the ground until the end of the mission. Finally, 13 days after completion of the experiment all cultures were simultaneously thawed and prepared for analysis.

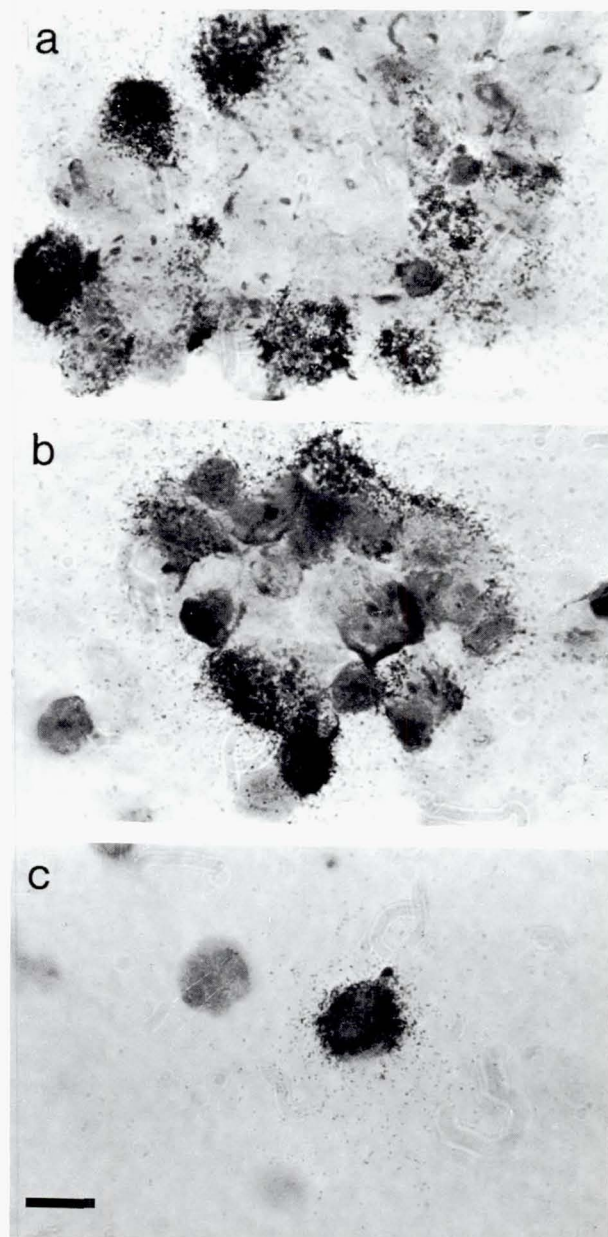
The main result is given in Fig. 1. The activation of the flight samples, measured as incorporation of tritiated thymidine into DNA, is less than 3 percent that of the ground controls (Fig. 1a). However, the cells survived the space flight, since the glucose consumption is only slightly lower in the flown than in the ground samples (Fig. 1b) and a significant number of radiolabeled nuclei are found by autoradiography of the lymphocytes exposed in flight to Con A (Fig. 2). The fact that glucose uptake seems not to be significantly altered by microgravity suggests that other membrane properties, such as binding of Con A and thymidine uptake, are also not dramatically changed. (The observation of lymphocyte aggregation in microgravity implies Con A binding to the surface membrane.) Many more nuclei are labeled in the activated cells at 1 g. Preliminary data from scanning and thin-section electron microscopy do not indicate significant structural differences between control and flown cells. Although the results are unequivocal, we note that they are from a single experiment and therefore need to be checked on future missions.

In the following discussion we assume that the constituents of cosmic radiation which can penetrate the culture flasks—that is, high-charge and high-energy particles such as iron nuclei—do not play a relevant role in this experiment. In fact, the probability that a significant number of resuspended cells are hit by the radiation in cultures containing 16×10^6 cells per flask is extremely low.

As discussed above, a decrease of lymphocyte reactivity was expected; however, the extent of the depression is surprising. Lymphocyte activation is triggered by at least two signals. In our experiment, the mitogenic signal is delivered specifically to T lymphocytes by Con A through its binding to glycosidic residues on the cell membrane followed by patching and capping. The second signal may be delivered by factors pro-

duced by macrophages (which are always present in lymphocyte cultures) and/or by subpopulations of T lymphocytes (interleukins). A third signal may be required and delivered through direct cell-cell contacts; although the finding of high activation in cultures diluted to as few as 5×10^4 cells per milliliter does not support the necessity of cell contacts. While the first two signals should reach the cell in microgravity, the third signal may be hindered since cell-cell contacts may be less probable in lymphocytes suspended at 0 g. However, the following considerations indicate that cell contacts must also occur in microgravity: (i) aggregates of cells, although less frequent, were formed in microgravity (Fig. 2); (ii) an experiment flown on the eighth space shuttle mission showed that contacts between cells and

Fig. 2. Light microscopic autoradiography of (a) ground and (b, c) flown lymphocytes exposed to Con A. Cells were fixed on glass slides with 5 percent trichloroacetic acid, rinsed with water for 24 hours, and coated with L-4 nuclear research emulsion (Ilford). After exposure for 7 days the slides were developed, fixed, and treated with Giemsa stain. Scale bar, 10 μm . (a) Most of the Con A-treated cells in the ground samples formed aggregates with labeled nuclei, as shown here. (b) Similar aggregates were formed in microgravity; however, their occurrence was markedly lower than at 1 g. (c) Single cells with labeled nuclei in the flown samples indicate that aggregation may not be necessary for activation.



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microcarrier beads are at least as effective in space as on the ground (unpublished results); (iii) passive cell movements in the medium, which may contribute to establish cell contacts, are not hindered by gravitational forces; (iv) calculations based on the volume of the flask and the cell concentration show that the average statistical distance between cells was less than 0.05 mm in our cultures; and (v) considering the various signals involved in activation, it is important to note that the comparative results in Fig. 1a are consistent with activation being an all-or-none phenomenon.

Although our observations are in agreement with the results found with lymphocytes taken from crew members after spaceflight, we cannot extrapolate the data derived from experiments in vitro to changes occurring in vivo. Experiments planned for the D-1 and Spacelab 4 missions in 1985 and 1986 should clarify the question of lymphocyte efficiency in space.

Considering what is presently known about the behavior of cells at different g values, we can see a relatively consistent picture into which our results from Spacelab 1 fit very well. At high g , cells divide faster at the expense of reduced motility, since energy consumption remains the same. In microgravity, lymphocytes show a dramatic reduction in proliferation rate, reduced glucose consumption, but a strong increase of interferon secretion. WI-38 embryonic lung cells, which differ from lymphocytes in that they do not undergo differentiation steps, grow and move normally at 0 g , but they also consume less glucose. In conclusion, most of the cells investigated appear to be sensitive to gravity; the effect seems to be stronger with cells such as lymphocytes, which are transformed by mitogens from a dormant to an activated state.

The results we have obtained so far have contributed to an increase in the knowledge of the influence of gravity on basic cellular mechanisms, to clarifying certain biomedical aspects of the effect of spaceflight on the immune system, and to developing useful biotechnological processes. Although the mechanisms involved in gravitational effects on cells are still unknown and a gravity sensor has not yet been identified, we can conclude on the basis of results to date that cells are sensitive to gravity.

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Gravity exerts a fundamental influence on Earth organisms. This will sound like a truism to many scientists, but the fact is that the importance of the gravitational force for life-sciences research was never really faced until the space age dawned. Some very basic physical processes work quite differently under normal Earth gravity (1 g) and under so-called 'microgravity' conditions. But it is not just that fundamental physical processes are different in microgravity: since these physical processes are involved in many biological processes, biological systems may also be altered or affected in new ways.

While it is conceivable that a complex organism like a human body experiences a number of effects in a microgravity environment, it is much more difficult to accept that a single cell may perceive gravity. Calculations based on the physical properties of the cell membrane, organelles and cytoskeleton seem to predict that no gravity effect may be expected at the cellular level. However, most of these considerations look at the cell as a static and unchangeable system, and do not account for the countless single events occurring in sequence or in parallel in a living cell during differentiation, proliferation and gene expression. All this machinery has developed under 1 g conditions, i.e. it is designed to make optimum use of gravity during differentiation, proliferation and gene expression. Evolution at 1 g has probably ruled out the unfavourable alternative pathways leading to failures in the cellular functions. The system did not have the chance to go through evolution at zero g and to exclude these unfavourable pathways.

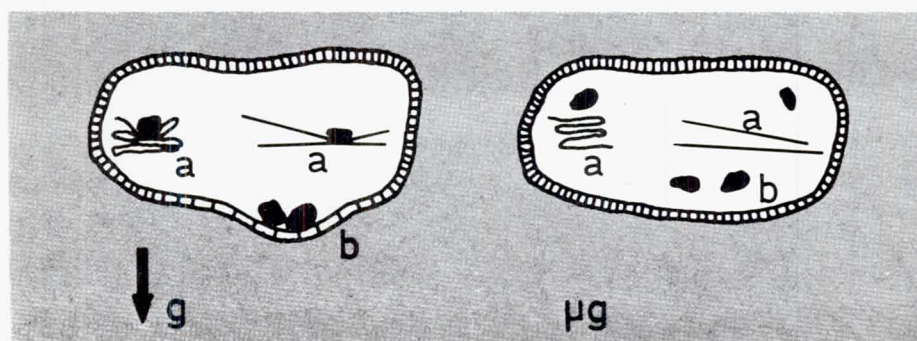
The discussion on microgravity effects in the context of cell biology presented below is by no means complete: only a few areas will be touched upon, but they have been chosen to demonstrate how microgravity will open up important new areas in the study of the life sciences.

Decreased pressure/strain in cells and organisms

An obvious effect of reduced gravitational force is that the physical pressures and loads in organisms and cells change. An example of how the physical strain on structures changes is given in Figure 5.1, from cellular biology. Of course, examples could just as well have been chosen at organism level: the pressure in the body as a whole changes under microgravity.

The reduction in pressure and strain will be of the utmost importance in many areas of both plant and animal physiology. Besides those involving human and animal physiology, which have been discussed in earlier chapters, we can note:

- studies of the consequences of changes in membrane stress
- studies of the shape or organism, gravimorphism, etc.
- studies of the cytoskeleton.



Introduction

Biophysical Aspects

Figure 5.1. Physical strains are different under 1 g conditions and in space, i.e. under microgravity conditions. To the left is illustrated, in a schematic and simplified way, how internal masses (a) can cause load and strain on internal cell structures; strain in cellular membranes can likewise increase due to cellular masses or cell content by itself (b). Such strains are more or less absent when the system is moved to microgravity, as shown in the sketch on the right.

Sedimentation of particles, buoyancy of bubbles, etc.

Another obvious, but fundamental effect of reduced gravitational force is that sedimentation of particles in fluids is diminished or even totally absent (Fig. 5.2). Correspondingly, gas-filled volumes, vesicles, etc. will not move as effectively, because of reduced buoyancy, or will simply remain in place under microgravity conditions.

The absence of sedimentation and buoyancy under microgravity will enable life scientists to carry out fundamental studies of:

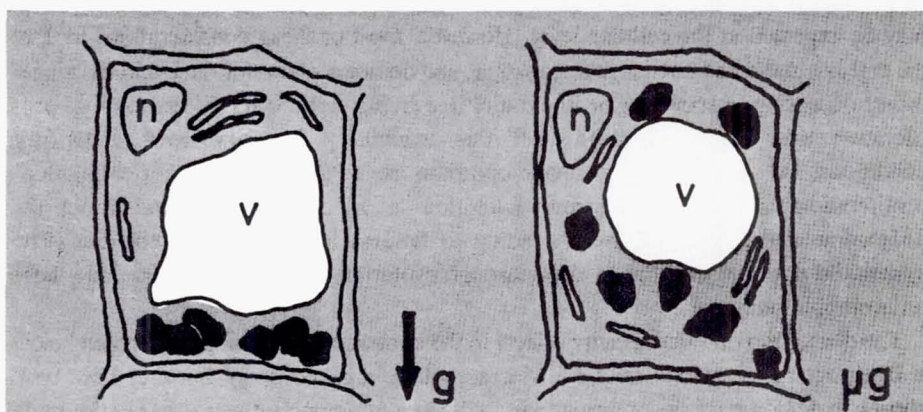
- the perception of gravity and forces
- the overall balance systems of plants, animals, man, etc.

Figure 5.2. Schematic of particle sedimentation in a plant cell. To the left, under 1 g conditions, the denser black particles have sedimented to the lowermost part of the cell.

To the right, under microgravity conditions, no settling occurs and the particles are almost evenly distributed in the cell volume.

n = nucleus;

v = vacuole



Stirring/convection

When density gradients are present in a liquid or gas under normal 1 g conditions, stirring and mixing take place. Because of this movement, the gradients ultimately disappear. A thermal gradient induces convection and mixing in the same way.

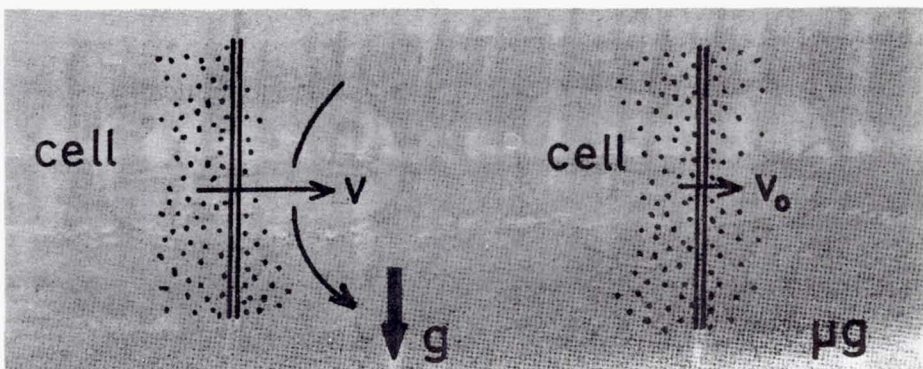
In microgravity, such mixing effects simply do not occur (Fig. 5.3). This absence of mixing is of interest not only to physicists and material scientists, but also to the life scientist, since studies under space conditions are likely to yield interesting results in many relevant areas in terms of:

- mixing processes in organisms
- diffusion-controlled processes
- limitations (in the absence of stirring) of the transport of material into and out of the cell through the cell membrane, e.g. by exchange of electrolytes, etc.
- other convection phenomena that may occur in microgravity.

Figure 5.3. Natural mixing and convection disappears under microgravity conditions.

To the left is a schematic of how molecules are transported over a membrane along a concentration gradient. The transport occurs with a certain flow velocity v , under 1 g conditions, Mixing due to density gradients, etc., influences the systems and introduces a 'stirring effect' which facilitates the transport.

To the right, mixing is absent in microgravity. Fewer 'stirring effects' are introduced and the transport of molecules across the membrane will be affected and slowed down compared to 1 g.



Changes of surfaces

Under normal 1 g conditions, gravity often inhibits increases in the size of bubbles and drops. Surface forces and phenomena depending on flow are easier to study under microgravity conditions. For example, the height of a liquid column in a capillary will be much greater in microgravity, because of the relative increase in surface tension in proportion to gravity (Fig. 5.4).

Several processes that must also interest the life scientist will therefore be affected in space:

- transport processes in organisms
- uptake processes by, for example, plants and roots
- growth processes as a consequence of the effects on transport processes
- transport in artificial soil materials, etc.
- all phenomena connected with the creation and existence of surfaces (including vesicles and membranes), etc.

Bioprocessing in space

The processes mentioned above may change mechanisms governing fundamental cellular functions. These alterations may be exploited for bioprocessing in microgravity. One of the tasks on future space missions will be the selection of biological systems suitable for bio-technological applications.

In addition, it is of great importance to the life scientist that the separation and isolation of biological specimens (molecules, particles, cells) is also effected. The key concept here is one of 'bioprocessing in space', and this will be dealt with in more detail in Chapter 9.

The absence of convection and sedimentation is crucial to many bioprocessing techniques that need to be investigated in space. These range from the very fundamental production of protein crystals for basic life-sciences research, to the delicate fabrication and separation of minute amounts of highly important medical substances. One of the most widely used analytical techniques is electrophoresis. It will be important to establish whether electrophoresis, in combination with the absence of convection in liquids under space conditions, might be used for the separation of biological materials in quantities and with purity levels unattainable on Earth.

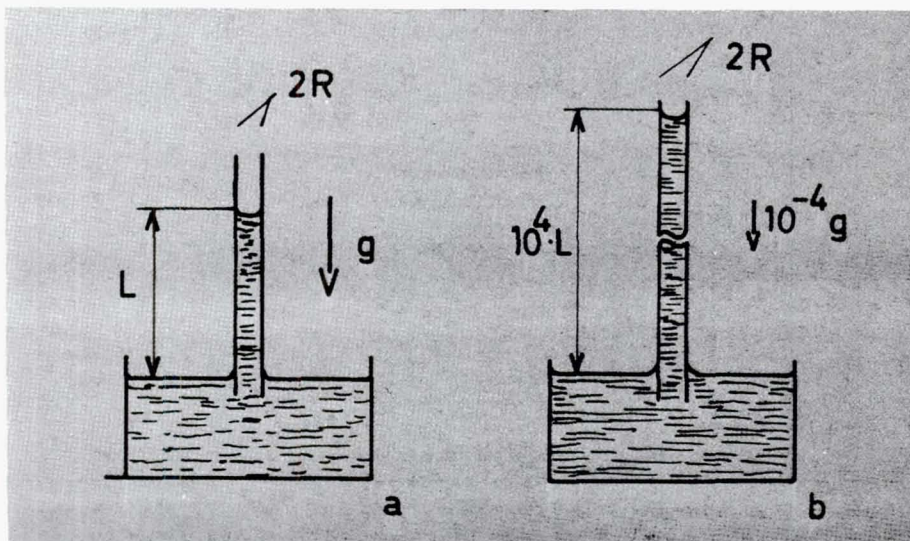


Figure 5.4. Rise of a liquid in a capillary under Earth and space conditions.

The importance of surface forces relative to hydrostatic forces will increase in space, where the latter are drastically reduced. An example is given here.

A liquid rising due to capillary forces to a height L in a capillary (diameter $2R$) at 1 g is shown in (a). It will rise to a much higher level when the g force is reduced.

To the right (b), the force is reduced to $10^{-4} - 10^{-3} g$ (a value expected in Spacelab), and the liquid column will now be 1000-10000 times higher if the diameter and the liquid remains the same.

What Do We Know Today? **Unicellular and mammalian cells**

The behaviour of single cells in space was first studied on the early biosatellites launched in the mid-sixties. Unfortunately, the lack of proper controls, and technical failures, often impaired the scientific credibility of early investigations.

Only a selection of the experiments that contributed significantly to the progress of cell-biology experiments in space are discussed here. The most interesting changes that have been observed in microgravity are summarised in Table 5.1. Historically, at least as far as US missions are concerned, two periods can be distinguished: the pre-Shuttle and the Spacelab/Shuttle eras. Most of the projects carried out in the first period have been reviewed earlier. A clear effect of space flight (i.e. microgravity and cosmic radiation) on bacterial growth was discovered in an experiment performed on Biosatellite-II in 1967. The objective of the investigation was to study the effect of radiation on the propagation of bacterial growth. Both organisms tested, namely *Salmonella typhimurium* and *Escherichia coli*, exhibited a significantly higher cell densities in space than in the ground controls.

A few years later, the effect of space flight was studied on WI-38 human embryonic lung cells in a tissue culture package — called 'Woodlawn Wanderer 9' — installed on board Skylab. This was probably the most sophisticated equipment for cell cultures ever used in space. The authors reported that no effect of microgravity was detected on the cells investigated. However, the significance of the marked reduction in glucose consumption has been ignored. In fact, this result clearly indicates that the metabolism of the cells was changed in microgravity. Changes in metabolism and in the cell cycle were discovered in a unicellular organism (*Paramecium tetraurelia*) during a flight on a Soviet mission. Moreover, the resistance of bacteria to antibiotics was increased

Table 5.1. Alteration of cellular functions in microgravity

Cell Type	Function Altered	Investigator
Human embryonic lung cells	<i>Energy consumption:</i> 20% reduction in glucose consumption	Montgomery
Human lymphocytes	<i>Biosynthesis:</i> 500% increase in interferon production	Bàtkai, Tàlas
	<i>Differentiation:</i> ^{1,2} 90% depression of activation by Con A	Cogoli
Escherichia coli	<i>Conjugation:</i> ¹ 400% increase in DNA transfer	Ciferri
	<i>Membrane permeability:</i> ^{1,2} Increased resistance to antibiotics	Tixador
Paramecium aurelia ² Chlamydomonas R. Bacillus subtilis Anis cells	<i>Cell proliferation:</i> ¹ Manifold increase in biomass yield	Planel Mergenhagen Mennigmann Theimer
Physarum polycephalum	<i>Intracellular convection:</i> ¹ Increase in frequency and velocity of cytoplasmic streaming	Briegleb

¹ Experiments with an in-flight 1-g control

² Results obtained from independent experiments on at least two different space missions

in space. In addition, experiments performed on two different space flights reported that the biosynthesis of interferon in-vitro is approximately five times greater in space.

An important step towards large-scale cultures of substratum-dependent mammalian cells was performed on the Space Shuttle 'Challenger' (flight STS-8) in 1983. The fact that cells adhere normally to Cytodex microcarriers, even though resuspended in microgravity, shows that no restriction is imposed on the culture of adhering cells in space.

The advent of Spacelab opened up access to biological experiments in microgravity to a broader community than in the past. In particular Biorack, a facility provided by ESA, hosted several successful experiments with single cells on the German Spacelab-D1 mission in 1985. These investigations have shown that single cells in cultures are sensitive to gravity, i.e. that important cellular functions are affected by low-g conditions. Due to their importance, these results are discussed in more detail below.

The effect of gravity on lymphocytes in-vitro has been studied extensively under microgravity in space, in simulated low-gravity in the clinostat, and in hypergravity. A synopsis of the results is given in Figure 5.5. Lymphocytes are easily purified from peripheral blood and can be activated chemically or by exposure to mitogens in culture. Mitogens are substances from different origins (many from plants) having the property of binding to components of the cell membrane (mostly to sugar moieties of membrane glycoproteins). When mitogens like Concanavalin A (Con A) interact with lymphocytes, T-cells undergo a series of changes (activation), leading ultimately to cell division (mitosis). T-lymphocytes secrete lymphokines (IL-2, IL-3, IL-4 as well as interferon). The mechanisms of the activation in-vitro are analogous to those occurring in-vivo when the immune system is challenged by antigens. The main difference is that antigen activation is based on clonal selection of a restricted population of cells, whereas mitogenic activation is polyclonal, i.e. involves all T- and/or B-lymphocytes. Altogether, lymphocytes are a good model for fulfilling the goals of investigations in three main areas of gravitational biology:

- basic science, i.e. how gravity affects differentiation processes
- biomedical diagnosis, i.e. how the immune system of astronauts adapts to spaceflight conditions

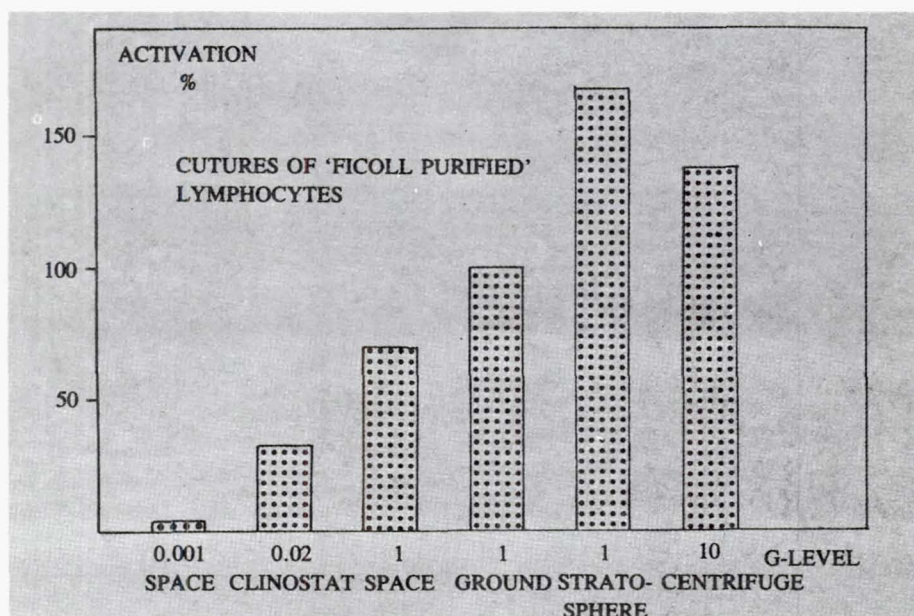


Figure 5.5. Summary of gravitation and cosmic radiation effects on T-lymphocyte activation by Con A. The results are expressed as percentages of the corresponding 1 g control. The increase at 10 g is due to the simultaneous activation of T- and B-lymphocytes.

- biotechnology, i.e. how the production of important substances (like interferon and interleukines produced by T-lymphocytes) may be qualitatively and quantitatively improved in space.

The results of three independent experiments showed that lymphocyte activation is depressed by more than 90% in space. Figures 5.6 and 5.7 compare the ultrastructural differences observed by transmission electron microscopy between cells cultured in space at zero g, and at 1 g, respectively.

Combining the results on mitogenic activation with those on interferon production, one may speculate that in microgravity lymphocyte proliferation is depressed in favour of the biosynthesis of an important product like interferon. This does not necessarily mean that plants for interferon production should be set up in space; rather lymphocyte behaviour in space can be considered as an example of a mammalian cell undergoing changes that may be of biotechnological relevance if detected in other cell systems.

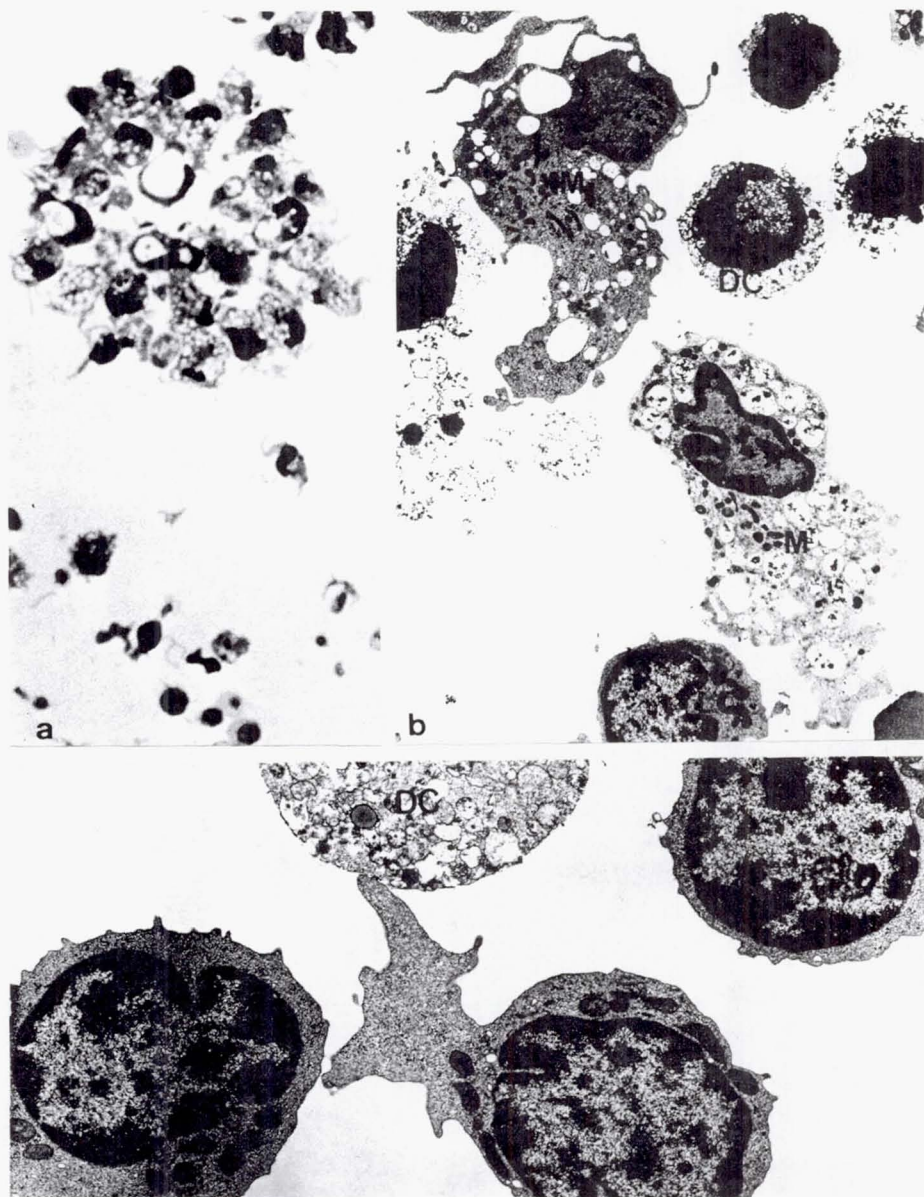


Figure 5.6. Light and electron micrographs of in-vitro cultivated cells under microgravity conditions. (Courtesy of O. Müller)

(a) Cluster of vacuolated macrophages (x1000).

(b) Two macrophages (M), dead cells (DC), and cellular debris and part of a normal lymphocyte (x5100).

(c) Three morphologically normal lymphocytes, one of which shows pseudopodia (*), and part of a degenerated cell (DC) at day 4 at zero g (x10000).

Plant cell biology studies

As mentioned previously in Chapter 4, the development of plants from single somatic cell cultures is likely to be a fruitful area of investigation under microgravity conditions.

The use of plant cell cultures in space biology dates back to the joint US-USSR studies on the Soviet Biokosmos missions, during which preliminary experiments were performed using carrot cells. On the unmanned satellite missions Kosmos 782 and 1129, it was investigated whether free carrot cells, because of their totipotency and capacity for organised development, could undergo embryogenesis under microgravity conditions. It was found that morphogenetically competent carrot cells could give rise to embryos and that the latter could further develop small plantlets in about 20 d in darkness. After flight, normal plants were grown to maturity from these samples.

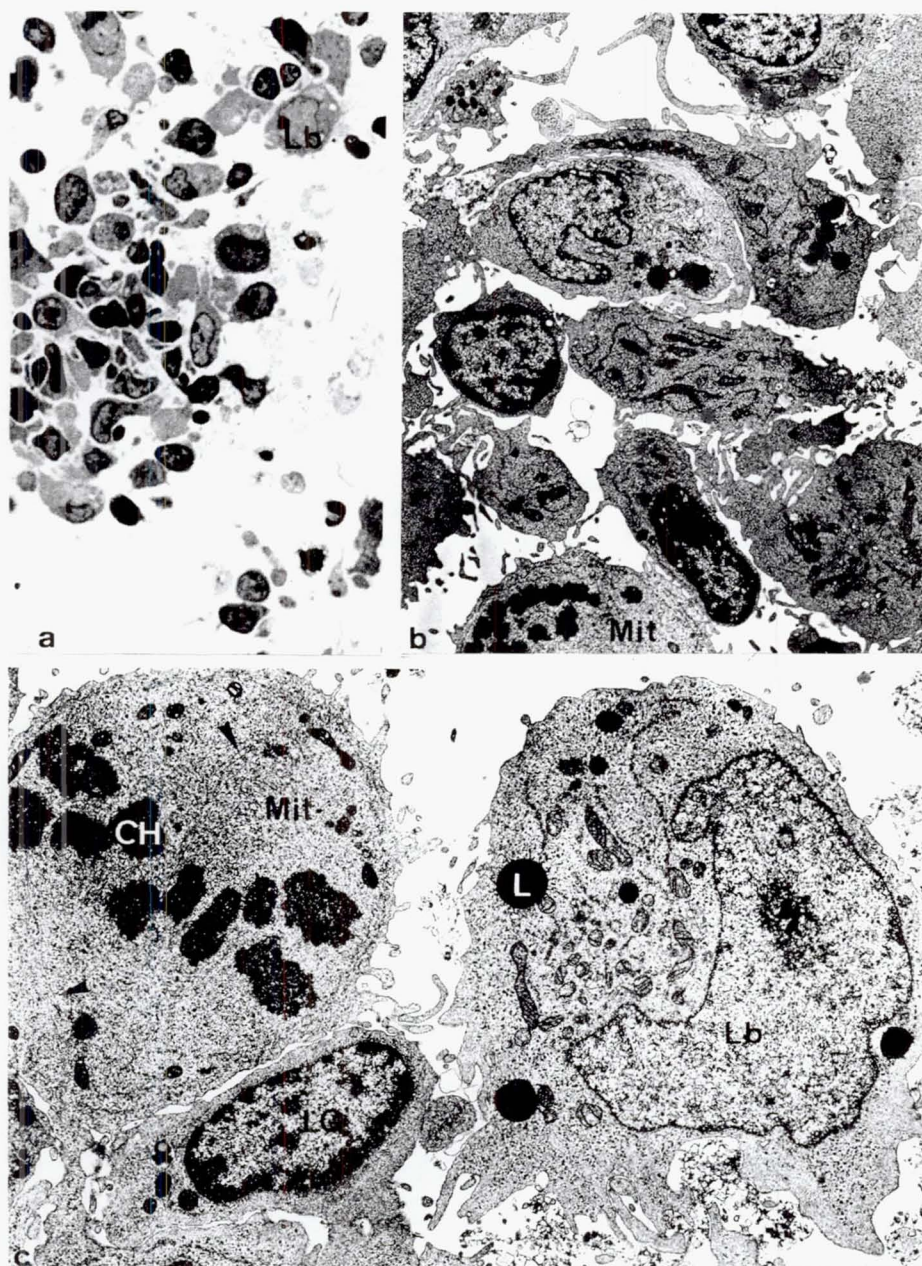


Figure 5.7. Morphology of in-vitro cultivated cells at 1g in the reference centrifuge on board Spacelab. (Courtesy of O. Müller)

(a) Light micrograph of a lymphocyte-lymphoblast (LC) aggregation at day 3 (x1000).

(b) Higher magnification (x4500) of aggregated cells, one being in mitosis (Mit.).

(c) A lymphocyte (LC), a lymphoblast (LB) with lipid droplets (L), and a lymphoblast in meta-phase showing chromosomes (CH) and spindle fibres (arrows) at flight day 4 (x8200).

In the domain of cell biology, plant cells can be subdivided into two types:

- unicellular plants
- protoplasts.

Unicellular plants

An experiment with the unicellular algae *Chlamydomonas reinhardtii* was performed in the Biorack on the Spacelab-D1 mission. The aim was to study the biological clock and other cellular functions such as swimming velocity, proliferation, viability and ultrastructural alterations. It appears that the circadian rhythm of the algae was not influenced by space flight. Similar experiments are planned for the Space Station. It is recommended that an algae colony be developed directly under microgravity, starting from single cells to form a colony that can then be cloned. From these clones, one particular colony should be selected and developed under constant temperature and light conditions until flagella have been formed. These cells should then be used for the experiments.

The most important problems that can be addressed using unicellular plant cells are:

- the correlation of motility and energy consumption
- survival rate
- ultrastructural studies.

Protoplasts

Protoplasts are plant cells whose cell walls have been removed enzymatically. Only a few experiments with plant-cell cultures have been carried out in space. However, investigations of this kind have been suggested, and a morphogenetic study on protoplasts has been accepted for Biorack on IML-1 and Biokosmos-9.

On the Space Station, it would be possible to pursue and extend studies using protoplasts and plant-cell cultures. The following experimental tissue culture systems may prove valuable in approaching the problem of gravimorphogenesis:

- protoplasts from higher plants, which can deposit new cellulose cell walls, divide, multiply, undergo organised development and ultimately give rise to embryos and mature plants
- free somatic cells from higher plants, which through division under sterile conditions in a nutrient medium may express morphogenic competence and form somatic embryos that develop into plants
- propagules with predetermined organs (root/shoots), or growing points developed to varying degrees.

Some of these systems, if not all, may well be adapted to the establishment of a gravitationally responsive unit. Whether higher plant cells in isolation respond to gravitational stimulus, or rather a certain degree of morphological complexity is required, is one of the questions that can be answered on a Space Station. It should also be possible to establish whether or not gravitational responsiveness varies with different degrees of organisation or stages of morphological complexity. Since the normal uptake mechanisms in higher plants are dependent on well-developed transport structures within the tissue, it is of interest to learn whether these structures develop from cell culture systems as well under microgravity conditions as they do on Earth. It is also yet to be established whether several consecutive 'generations' of plant cells in culture (protoplasts, somatic embryos, suspension cells) can differentiate to mature plants in space.

The use of plant-cell cultures may also produce spin-offs in space plant

biotechnology. The production of secondary metabolites of economic importance may be modified under microgravity.

Chronobiology

here is some evidence, both from clinostat studies and from the 1983 Spacelab-1 (STS-9) flight, that the *Neurospora crassa* experiment revealed a marked reduction in the amplitude and clarity of the conidiation rhythm in some samples after 6 d. However, the biological rhythm of simple algae cells in Biorack on Spacelab-D1 appeared to be totally undisturbed. These contradictory results merit further investigation.

One of the questions still to be answered is whether circadian rhythms in cells are ruled by an exogenous 'Zeitgeber', i.e. some extracellular time signal. The experiment in Biorack with the algae *Chlamydomonas* addressed this problem. Microgravity may influence certain time-dependent biological processes in the single cell. For instance, the cell cycle, i.e. the sequence of events between two cell divisions, ranges from 15 to 24 h for an animal cell, and between two and four days for plant cells, with a characteristic duration for each type of cell. Lymphocytes require 72 h of incubation after exposure to the mitogen Con A for maximum activation. Cells from rape seeds take three days for one division cycle. Since it is conceivable that certain dynamic cell functions, such as cytoplasmic streaming, are influenced by gravity, it is of primary importance to assess the effects of space on biological clocks.

At least two fundamental questions can be asked about circadian rhythms in a microgravity environment:

- What is the effect of eliminating 24 h signals from subtle environmental parameters such as the Earth's magnetic field?
- What is the effect of microgravity on the rhythms in unicellular and multicellular organisms?

The 1 g reference centrifuge in Biorack allows these two effects to be distinguished.

Most important of all is the fact that the fundamental mechanisms of the circadian oscillating system has not yet been determined in any organism. If it can be established that circadian rhythms persist in a space laboratory circling the Earth every 90 min, and that the period of the rhythm is not an exact multiple of 90 min (so as to rule out the hypothesis that there is frequency demultiplication), then the problem of the effect of microgravity on the basic mechanism can be investigated. Since the generation of the rhythms is likely to involve the intracellular compartmentalisation of metabolically active compounds, and thus membrane transport systems, gravity may well affect the rhythms if there is disruption of subcellular organisation.

The first Biorack flight on the German Spacelab-D1 mission yielded very interesting scientific results, which in all probability will serve as a foundation for many further studies. The experiments performed with single cells will be discussed here. Preliminary reports on the complete set of experiments have been published in a special issue of *Naturwissenschaften*. A detailed report on Biorack and on the results of all experiments has been issued recently by ESA as Special Publication SP-1091.

Experiments with bacteria

Bacteria can be considered the simplest form of life on Earth; they are single cells

Results of Biorack Experiments on Spacelab-D1

not much longer than one thousandth of a millimetre, and they perform a great variety of essential functions in nature, without which the life chain could not exist. Under favourable conditions, bacteria proliferate rather rapidly by repeated cell division. Application of antibiotics can stop these processes and even destroy the bacteria.

It was shown in two Biorack experiments that microgravity has a stimulating effect on the proliferation of bacteria (*E. coli* and *Bacillus subtilis*). A third experiment suggests that space conditions make bacteria (*E. coli*) more resistant to antibiotics. Not only are these results scientifically interesting, they also suggest that humans are at greater risk in space than on Earth, given that there may be larger populations of bacteria, which are, moreover, less sensitive to antibiotics. This consideration is even more important in the light of the results of two other Biorack experiments, to be discussed later, which indicate that the human defence mechanism is weakened in space.

In some bacteria, two complementary cells meet and establish a physical bridge through which the DNA molecules that carry all genetic information are exchanged. The process, known as 'conjugation', yields cells with so-called 'recombinant DNA'. In modern genetic engineering, recombinant DNA is produced by the artificial introduction of isolated genes into bacteria. Bacteria with recombinant DNA produce compounds that are coded by the introduced gene and harvested from the culture. In this way — to quote a classical example — insulin can be obtained from bacteria whose DNA has been recombined with the human gene for insulin. Clearly, this technique has enormous potential for commercial application. In one Biorack experiment, inconclusive results were obtained concerning the artificial introduction of isolated genes into bacteria (*E. coli*). However, it was shown that transfer of DNA from one bacterium to another in the conjugation process is three to four times more extensive in microgravity than under 1 g conditions. This highly interesting result provides the first evidence of gravity effects at hitherto unexpected subcellular levels.

Some bacteria are able to overcome harsh environmental conditions, such as lack of nutrients, by changing into a dormant organism, the spore. This sporulation provides an excellent yet 'simple' model for the study of cell differentiation; the acquisition by the cell of different functions. In embryonic development, originally similar cells differentiate, for example, into skin cells and nerve cells that have totally different functions. A special case of differentiation occurs when a normal cell becomes a cancer cell and begins uncontrolled proliferation.

A number of Biorack experiments have addressed the question of differentiation. In the experiment with bacteria (*Bacillus subtilis*), the results suggest that sporulation is strongly retarded, if not inhibited, under space conditions.

To conclude with the bacteria experiments, it has been shown in Biorack that microgravity also affects the simplest and smallest forms of life. This result is not only important for the study on bacteria themselves, but has potential significance for the behaviour of all higher organisms in space, including man.

Experiments with unicellular organisms

Besides bacteria, there are many other organisms that also consist of a single cell. This cell is, however, of another, more complex type, the eukaryotic cell. In addition to having a distinct nucleus, the site of the molecules carrying the cell's genetic code, it contains many small bodies called 'organelles', with a variety of specialised functions. Such unicellular organisms occur in soil and ponds, and in vast numbers in our oceans (plankton), and are members of both the plant and animal kingdoms. They can vary in size from approximately ten times the size of a bacterium, to as much

as several centimetres in length, and they can perform a variety of functions reminiscent of those of true plants and animals. Moreover, like bacteria, these organisms can proliferate through repeated cell divisions, when sufficient nutrients are available.

Two Biorack experiments showed that, in line with the effect found on bacteria, microgravity strongly enhances cell proliferation in the algae *Chlamydomonas* and in the protozoan *Paramecium*. In *Paramecium* cell numbers four times higher than those obtained under 1 g conditions were found (Fig. 5.8). As mentioned above, the biological periodicity in *Chlamydomonas* appeared to be totally undisturbed under space conditions and thus, in this respect, the cells appear to function normally. However, they did undergo cell divisions at a faster rate.

A third experiment in this series studied the behaviour of a slime mold called *Physarum polycephalum* which, although it can grow to many centimetres in diameter, consists of one giant cell with millions of nuclei. An elaborate network of veins maintains a continuous flow of protoplasm, the direction of which reverses periodically. Also this experiment shows that normal periodicity was maintained. However, the velocity of protoplasmic streaming increased in microgravity. As with the other two experiments, this result may be interpreted as a speeding up of the processes of life at cellular level: normal internal rhythms are maintained, but proliferation rate is increased.

Experiments with human cells

Isolated single cells from human tissue such as organs, skin or blood, can be cultivated and studied in the same way as unicellular organisms. Two Biorack experiments have shown that microgravity completely inhibits lymphocyte activation, thus confirming the results obtained in Spacelab-1. The investigations were carried out on cultures of lymphocytes prepared on the ground and tested in space, and with whole-blood samples taken from the crew and tested in flight, respectively. In addition to the interest in the effect of microgravity on basic biological mechanisms, it also important to assess the health risk arising from an impairment of the efficiency of the immune system.

In conclusion, it is highly desirable that several, if not all, of the experiments mentioned be repeated in space. The biological importance of the outcome certainly justifies seeking the best statistics possible.

This chapter started with a simple review of biophysical effects and phenomena that differ under normal gravity conditions and in microgravity. They are, therefore, important to bear in mind when considering studies of cellular processes in space.

In some cases, the biophysical effects mentioned describe sensor functions, as in the case of 'balance organs' of plants and animals. In such cases, sedimentation of denser particles in individual — specially organised — cells provides the fundamental basis of the ability to recognise a reference direction. In most instances the reference direction is, obviously, that of the gravity vector. Evolution on Earth has certainly favoured organisms that are constructed to withstand and take advantage of the gravitational force.

It is worthwhile looking at an arbitrary cell system and discussing, with the above remarks in mind, the interaction between the cell components and gravity. Figure 5.9 shows a hypothetical unicellular organism, but the same considerations apply to a multicellular one also.

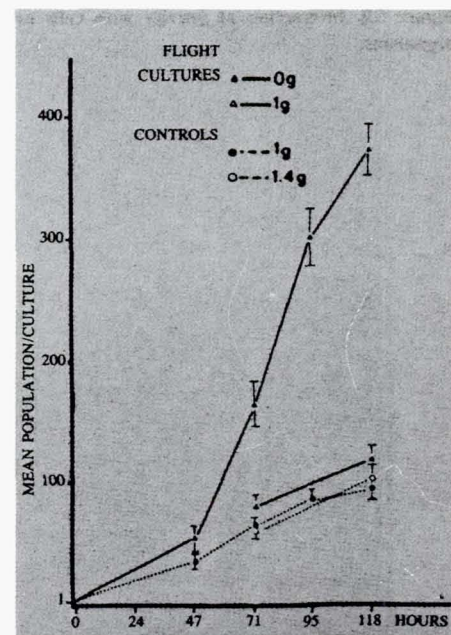
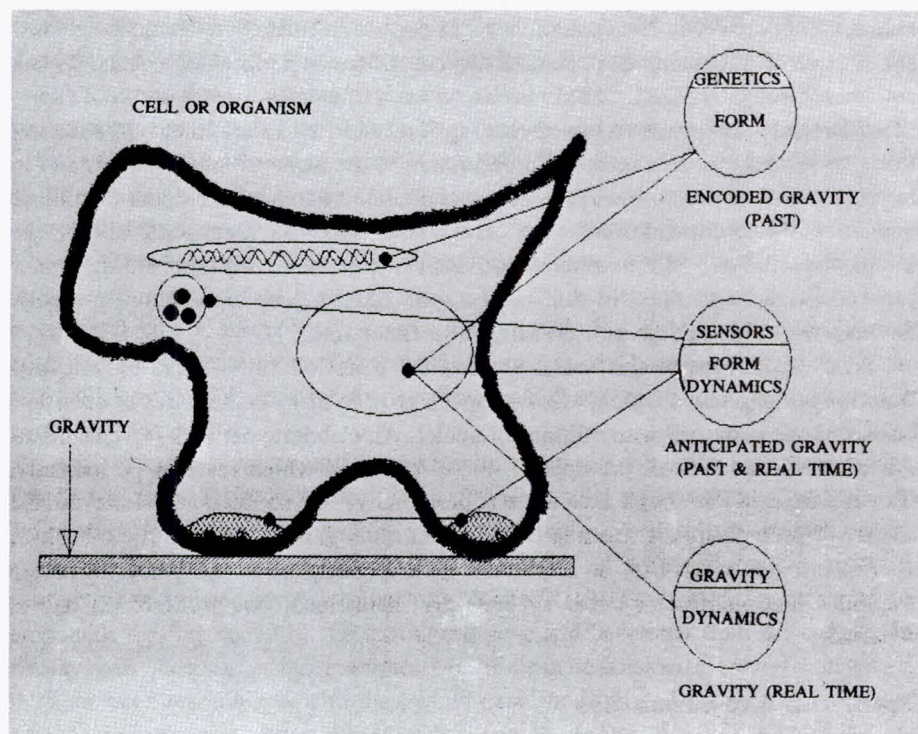


Figure 5.8. Population growth curves of in-flight and control cultures of *Paramecium* fixed at different times.

How Cells Could be Influenced by Gravity

Figure 5.9. Interaction of gravity with cells or organisms.



Gravity may act on at least three 'levels':

- through genes
- through sensors
- through molecular interactions.

Gravity acting through genes

During evolution, the ever-present force of Earth's gravity has been 'incorporated' into genes that determine the form and function of the organism. The basic form of plants and animals has been adapted to the environment the organism will be living in, and to the function it has to perform. In a sense, the three-dimensional morphology can be said to have been encoded in the genes through past interaction with gravity; it can be termed 'encoded gravity'. One would not expect this action of gravity to change over a few generations under microgravity conditions, since there is no reason to believe that the Darwinian theory of random mutations and selection would favour an acceleration of changes in encoded gravity. Thus, the basic form of a particular organism will, in all likelihood, be maintained even in a low-gravity environment.

However, expression of an organism's genom requires proper functioning of the appropriate biological machinery. If, for some reason, the machinery itself were inhibited by lack of real-time gravity, then expression would be impaired and no pre-established form could be generated.

Gravity acting through sensors

Sensors in this context are considered to be biological systems that provide real-time information about gravity. Information may be obtained directly from specialised structures, such as the vestibular organ in vertebrates or the statocytes in plant root caps.

It is important to point out that at the cellular level, the question of sensors is a rather complex one. If a living cell were to have a substructure with sedimentary heavy particles, that structure could be hypothesised to be a sensor organelle. However, the tension in structural cellular networks (e.g. the microfilament system) may itself somehow provide information to the cell about the gravity vector.

If the cell would really possess a gravity sensor organelle(s), then cellular network tension could well contribute to, or be mediative in, the act of sensing a changing gravity vector.

A similar remark can be made concerning gravity-mediated changes in energy requirements (also discussed earlier in this chapter). The energy spent by cells under 1 g conditions to maintain their positional homeostasis can be estimated. Changed levels of gravitation may change the cell's energy requirements and could hence provide information about gravity. It is argued that such a system could substitute for a real gravity-sensor organelle. As mentioned above, this is true in an integrated system that includes sedimentary particles giving positional information relative to the gravity vector.

Sensors generate the necessary feedback data to establish the precoded form of an organism successfully, to adapt the organism to changes in the environment and to maintain a proper balance. This can be called 'form dynamics'; they anticipate the force of gravity. Their very existence, however, is an expression of encoded gravity. Thus, anticipation of real-time gravity is performed by systems developed from past gravity environments.

It should be mentioned that both morphogenesis and sensor functions are dependent on appropriate biological machineries that, if themselves dependent on real-time gravity, may interfere with the system under study.

Gravity acting through molecular interactions

Past-time gravity information encoded in genes contributes to the form and function of an organism, while real-time gravity information obtained via biological sensors contributes to the form-dynamics of an organism. These contributions can only be made through complex biological systems, which ultimately are based on highly regulated molecular interactions. Interference with these interactions will obviously cause interference with the form dynamics discussed.

In earlier publications, a hypothesis has been described to explain gravity effects on cells. This hypothesis proposes that microgravity causes minute deviations in the cellular machinery which, due to a frequent occurrence in active cells, accumulate to produce measurable changes in the cell. Two possibilities for the occurrence of such small deviations were given: one assumes a change in the cellular structural network, and consequently a change in the cell's energy requirements; the other directly addresses the above-mentioned molecular interactions and their sensitivity to gravitational fields.

Systems that are thermodynamically far from equilibrium and non-linear, such as enzymatic reaction chains with positive or negative feedback, may show quite unexpected behaviours, depending on changes in relevant system parameters. For example, in such a system, an increase in the concentration of some chemical constituents to a certain threshold value may cause it to oscillate steadily in time and/or in space, or it may drive the system to a point where a choice can essentially be made between two different steady states. At such a point (a so-called 'bifurcation point'), the intrinsic choice is purely a matter of chance. However, the system becomes extremely sensitive to external forces, allowing it to enter one of two possible specific states. The point

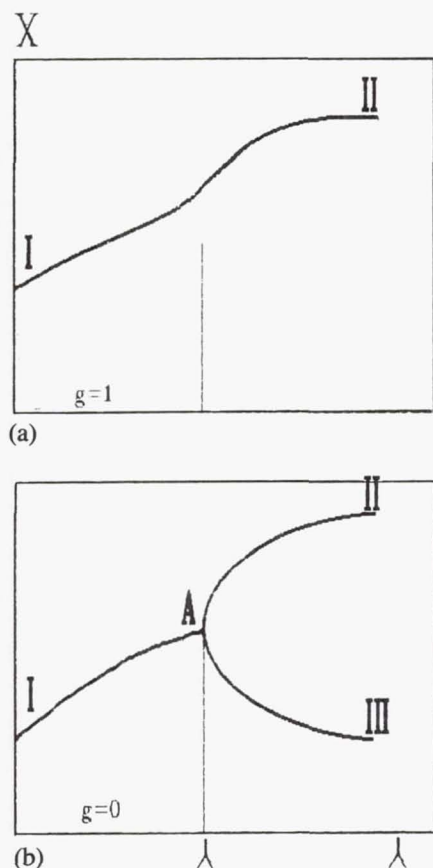


Figure 5.10. Biological pathways followed in response to the increase in a relevant system parameter (e.g. a growth factor, or a cofactor, or any other component specific to the system). Under normal gravity conditions (a) the pathway, which leads from I to II, has developed during evolution to a system which always functions correctly under Earth conditions. However, the system could appear to undergo bifurcation in the absence of gravity (b). Then a point A would exist at which the pathway, starting from I, can either lead to II or to III. III may not be meaningful to the biological system and the occurrence of it may drive the system into a non-functional state. (Ref. I. Prigogine & I Stengers, 1984)

has been made that relatively weak forces, like Earth's gravity, may have such pronounced effects on the subcellular systems as those discussed here.

The bifurcation phenomenon and its manifestation in biological cells would be of particular interest to gravitational biologists. It is tempting to speculate that in cells, particular molecular processes follow steady pathways that are determined by gravity. If so, microgravity could cause these processes to follow pathways beyond a presumed bifurcation point that are either 'normal', or different, driving the system into a different steady state, depending on the intrinsic choice made (Fig. 5.10).

In other words, a microgravity environment may cause some biological molecular interactions to go the 'wrong' way. Accumulations of such deviations may drive cells or multicellular systems into a non-functional state. Thus, it might be that biological systems apply the force of gravity so as to ensure that biologically meaningful pathways are followed.

Confirmation of such direct effects of gravity on the molecular interactions of biological systems would be an important scientific result in space biology.

Conclusions

Referring to Figure 5.9, we have discussed:

- past-time influences of gravity, which become encoded in genes and contribute to the form of an organism
- anticipated influences of gravity, which contribute via sensors to the form dynamics of an organism
- real-time influences of gravity, acting directly on the molecular interactions of the biological machinery.

The action of gravity via genes and sensors probably has much more relevance for multicellular organisms than for single cells. In other words, multicellular bodies are probably well suited to anticipating changes in the gravity vector. A distinction could be made between a particular effect of gravity on single cells being attributed to the action of sensors, or to direct action of gravity on molecular interactions (cell dynamics), by taking into account the presumed features of both actions, as shown in Table 5.2. The most important difference will be in the reaction time to changed gravitation and in the reversibility of the effect. Whereas sensor activity would show short reaction times and reversibility, action on cellular dynamic systems would be a relatively slow process yielding effects that may not be readily reversible.

In considering multicellular organisms, it is important to realise the contribution of single cells: (i) most organisms start their lives as a single cell, and (ii) most animals contain freely moving living cells (like white and red blood cells) as an essential part of their bodies. Thus, although cells in tissues of a multicellular organism are part of a high-level organisation that may prevent (or correct) malfunctioning of individual cells, free-moving or circulating cells are probably much less controllable. Direct action of gravity on the dynamics of these cells may therefore still influence the whole multicellular body.

It is important to point out that those effects of microgravity on cells that are based on processes like cellular network dynamics and energy transformation can also be revealed by the application of hypergravity. However, processes involving bifurcations can only be detected in microgravity. This basic fact supports the need for simultaneous investigations under microgravity, 1 g, as well as hypergravity.

Given the occurrence of bifurcations in far-from-equilibrium steady-state systems,

Table 5.2. Presumed differences in action of microgravity on cells if mediated via cellular dynamic systems or via sensor

	Cell Dynamics	Sensor Activity
Effect shown after:	Long exposure to microgravity	Short exposure to microgravity
Effect in:	Active cells only	Active and inactive cells
Effect:	Not readily reversible	Reversible
Result:	'Microgravity cell'	Normal cell sensing microgravity
Result:	Unpredictable behaviour	Programmed function

it therefore seems worthwhile adding a new field to space biology and studying the influence of microgravity on isolated, i.e. cell-free, biological processes. Some of these processes, e.g. assembly/disassembly of cytoskeletal proteins, when decoupled from the control of cellular machinery, might become more sensitive to a reduced-gravity environment. Moreover, many experiments designed to investigate this last aspect probably need only a few minutes of microgravity and, therefore, can be performed on a variety of spacecraft and rockets, e.g. sounding rockets.

One of the most important objectives of cell-biology research in space will be identification of the cellular mechanisms sensitive to gravity, as discussed above.

There are also important biomedical questions to be answered: the culturing and analysis of blood cells from crew members may be useful for health monitoring, particularly during longer flights and aboard the Space Station. The development of inflight 'diagnostic technology' is therefore of primary importance for the manned space flights of the next decades.

Some of the findings concerning the adaptation of single cells to microgravity may yield important spin-offs for biotechnology. However, an extensive programme of basic research is required before the advantages of biotechnological applications can be assessed. To carry out such a programme, a space-qualified bioreactor, i.e. an apparatus for large-scale culturing of cells, with automated change-of-medium, pH and oxygen controls, and automated cell harvesting should be developed.

In summary, this chapter has essentially discussed three kinds of biological experiments in space that have high 'space relevance': basic cell research, biomedical diagnostics, and biotechnology. Together with other biological fields treated in this report, cell biology will certainly see important new information emerge as a result of space experiments in the decades to come.

Summary of Scientific Objectives

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Head-down bed rest impairs vagal baroreflex responses and provokes orthostatic hypotension

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CONVERTINO, VICTOR A., DONALD F. DOERR, DWAIN L. ECKBERG, JANICE M. FRITSCH, AND JOAN VERNIKOS-DANELIS. Head-down bed rest impairs vagal baroreflex responses and provokes orthostatic hypotension. *J. Appl. Physiol.* 68(4): 1458-1464, 1990.—We studied vagally mediated carotid baroreceptor-cardiac reflexes in 11 healthy men before, during, and after 30 days of 6° head-down bed rest to test the hypothesis that baroreflex malfunction contributes to orthostatic hypotension in this model of simulated microgravity. Sigmoidal baroreflex response relationships were provoked with ramped neck pressure-suction sequences comprising pressure elevations to 40 mmHg followed by serial R-wave-triggered 15-mmHg reductions to -65 mmHg. Each R-R interval was plotted as a function of systolic pressure minus the neck chamber pressure applied during the interval. Compared with control measurements, base-line R-R intervals and the minimum, maximum, range, and maximum slope of the R-R interval-carotid pressure relationships were reduced ($P < 0.05$) from bed rest day 12 through recovery day 5. Baroreflex slopes were reduced more in four subjects who fainted during standing after bed rest than in six subjects who did not faint (-1.8 ± 0.7 vs. -0.3 ± 0.3 ms/mmHg, $P < 0.05$). There was a significant linear correlation ($r = 0.70$, $P < 0.05$) between changes of baroreflex slopes from before bed rest to bed rest day 25 and changes of systolic blood pressure during standing after bed rest. Although plasma volume declined by ~15% ($P < 0.05$), there was no significant correlation between reductions of plasma volume and changes of baroreflex responses. There were no significant changes of before and after plasma norepinephrine or epinephrine levels before and after bed rest during supine rest or sitting. We conclude that a model of microgravity, 6° head-down bed rest, significantly reduces the responsiveness and buffer capacity of vagal baroreflex-cardiac reflexes. Impaired baroreflex function appears to reduce the ability of subjects to adjust to transient changes of blood pressure during standing, and this may contribute to orthostatic intolerance.

sympathetic; blood pressure; baroreflex sensitivity; neck chamber

ASTRONAUTS who have been exposed to weightlessness have exhibited some degree of orthostatic intolerance, as manifested by reduction of mean arterial pressure and excessive cardioacceleration during standing postflight (4). Mechanisms underlying postflight orthostatic intolerance are unclear. Reduced blood volume contributes to, but probably is not a sufficient explanation for, orthostatic hypotension after spaceflight (3). Another pos-

sibility is that impaired baroreflex function after reentry impairs hemodynamic adjustments to standing.

Prolonged 6° head-down bed rest has been used to simulate hemodynamic changes that occur when humans are exposed to microgravity (3, 7, 8, 23). We conducted the present study to determine whether 1) there are alterations in the carotid baroreceptor stimulus-cardiac reflex response relationship after prolonged 6° head-down bed rest and 2) changes in baroreflex function (if they occur) are related to blood pressure responses during standing after bed rest. Our results indicate that impairment of vagal baroreflex function occurs during head-down bed rest and is associated with impairment of hemodynamic adjustments to standing and support the notion that baroreflex impairment may contribute to orthostatic hypotension after spaceflight.

MATERIALS AND METHODS

Subjects and measurements. Eleven healthy nonsmoking normotensive men [mean 38 ± 2 (SE) yr, range 30-45; mean height 179 ± 2 cm, range 173-188; mean weight 79 ± 2 kg, range 67-93] gave written informed consent to participate in this study, which was approved by the Kennedy Space Center Human Research Review Board. Selection of subjects was based on normal clinical results of a screening evaluation that comprised a detailed medical history, physical examination, psychological tests, complete blood count, urinalysis, 3-h glucose tolerance test, chest X-ray, resting and treadmill electrocardiograms, and a panel of blood chemistry analyses. No subject was taking medication at the time of the study. The regular daily activity levels of the subjects varied considerably, from sedentary to running 5 miles/day. During a 3-wk orientation testing period that preceded the study, all subjects were made familiar with the laboratory, the protocol, and the procedures.

Protocol. The experimental protocol was comprised of a 9-day ambulatory control period (C) followed by 30 days of 6° head-down bed rest (BR) and 5 days of recovery after bed rest (R). During bed rest, the subjects remained head-down without interruption. No conventional exercise was performed by the subjects during bed rest. However, during bed rest, three subjects received electromyostimulation to one leg for 20 min twice daily in a 3-day on 1-day off pattern as part of a supplemental

experiment on skeletal muscle. The baroreflex data of these subjects were combined with those of the remaining eight subjects, since there were no significant differences between the subject subgroups in the changes in baroreflex, plasma volume, catecholamines, or posture test responses.

During the 44-day experimental period, subjects lived 24 h/day in the Human Research Facility at NASA-Ames Research Center and followed the same controlled diet. The average daily caloric intake was 2,500–2,800 kcal (45% carbohydrate, 38% fat, 17% protein). Dietary sodium and potassium were held constant at ~120 and 60–80 meq/day, respectively. Fluid intake was ad libitum but restricted to 2,000 ml/day. The photoperiod was 16 h of light and 8 h of darkness with lights on at 0700. The 30-day bed rest period was chosen because it represents the projected minimum duration of future Space Station missions. The 6° head-down position was chosen because actual flight changes in some cardiovascular responses are closely simulated by this ground-based model (7, 23).

Each subject underwent a carotid-baroreflex test on the 4th day before bed rest (C4), on days 1, 3, 12, and 25 during bed rest (BR1, BR3, BR12, and BR25), and on days 2 and 5 of recovery (R2 and R5). In addition, the subjects returned to the laboratory after 25 days of uncontrolled recovery (R30) for a final baroreflex test. During the pre-bed-rest control and post-bed-rest recovery tests, a 30-min supine rest period preceded each session.

Baroreflex stimuli. Carotid baroreflex-cardiac reflex responses were measured with a method described previously (27). Briefly, a stepping-motor-driven bellows was used to deliver a series of pressure and suction steps to a Silastic neck chamber. During held expiration, a pressure of ~40 mmHg was delivered to the chamber and held for ~5 s; then, with the next R wave, the pressure sequentially stepped to ~25, 10, -5, -20, -35, -50, and -65 mmHg and then returned to ambient pressure. Pressure steps were triggered by R waves so that neck chamber pressure changes were superimposed on naturally occurring carotid pulses. This timing was chosen so that experimental baroreceptor stimuli would be as physiological as possible. With this technique, arterial pressure changes are small (27). During each test session the stimulus sequence was repeated seven times, and the data were averaged for each subject. Unpublished data indicate that baroreceptor stimulus-sinus node response relationships, measured in this way, are highly reproducible.¹ Blood pressures were measured with a sphygmomanometer at the beginning of each test session. R-R intervals for each pressure step were plotted against carotid distending pressures (systolic pressure minus neck chamber pressure applied during the heart beat).

We used R-R intervals to characterize the pressure input-neural output relationship. This usage is based on the relationships that exist between R-R intervals and

vagal-cardiac nerve activity and heart rate. R-R intervals are highly linear functions of vagal-cardiac nerve activity (17, 21). Because of this linear relationship, it is possible to use changes of R-R intervals as surrogates for changes of vagal-cardiac nerve activity and to compare responses before and after interventions such as bed rest, which alter base-line R-R intervals. Heart rates are calculated reciprocals of directly measured R-R intervals. Because the relationship between heart rate and vagal-cardiac nerve activity is curvilinear, it is extraordinarily difficult to compare responses to forcings when base-line heart rates are different. Unpublished data (D. L. Eckberg and J. M. Fritsch) indicate that R-R interval responses to the neck pressure sequence we used are not reduced by β -adrenergic blockade but are nearly abolished by muscarinic blockade. Therefore, our study focuses primarily on the vagal limb of the baroreceptor reflex.

Plasma measurements. Resting plasma volume was measured on C4, BR3, BR12, and BR25 with an Evans blue dye technique and was determined on R2 from changes in hematocrit and hemoglobin concentrations (14). Resting norepinephrine and epinephrine were measured on C4, BR1, BR12, BR25, and R1 with a radioenzymatic assay (26).

Posture tests. Posture tests were conducted before bed

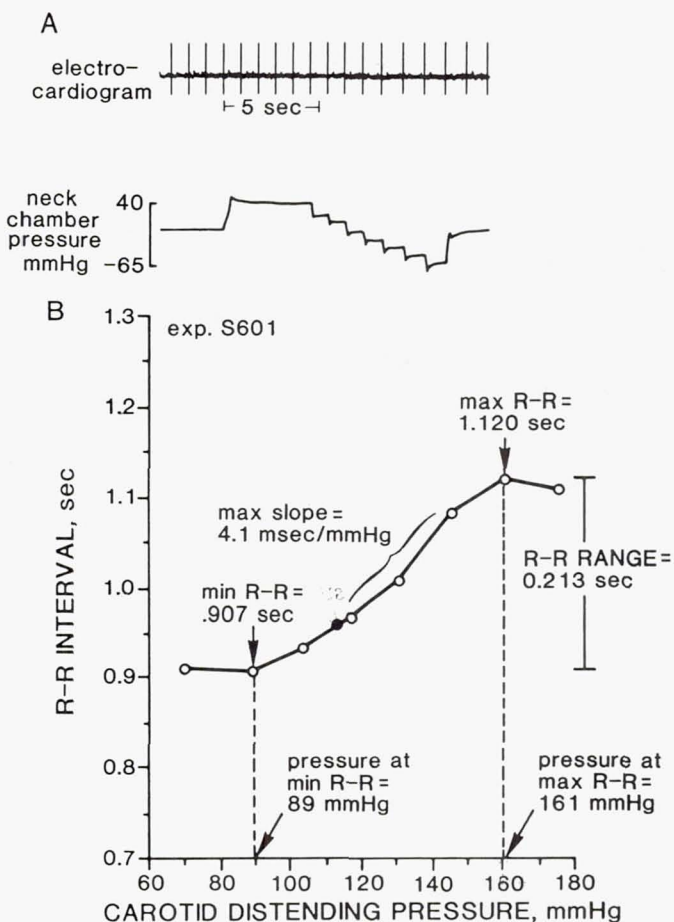


FIG. 1. Experimental record (A) and average response of 1 subject to 7 neck pressure sequences (B). B: parameters of baroreflex relationship used for analysis. Carotid distending pressure was considered to be systolic pressure minus neck chamber pressure. In this subject, steepest slope lay between the 4th and 6th points on relationship. ●, Position of operational point (base-line R-R interval).

¹ We measured carotid baroreceptor stimulus-sinus node response relationships on two occasions in 27 healthy subjects. The first relationship was virtually identical to the second, which was obtained 7–10 days later. Both maximum slopes and R-R interval ranges were comparable on the two occasions. Slopes averaged 5.7 ± 0.8 and 5.7 ± 0.7 ms/mmHg ($P = 0.93$, paired t test) and R-R interval ranges averaged 199 ± 21 and 210 ± 17 ms ($P = 0.36$).

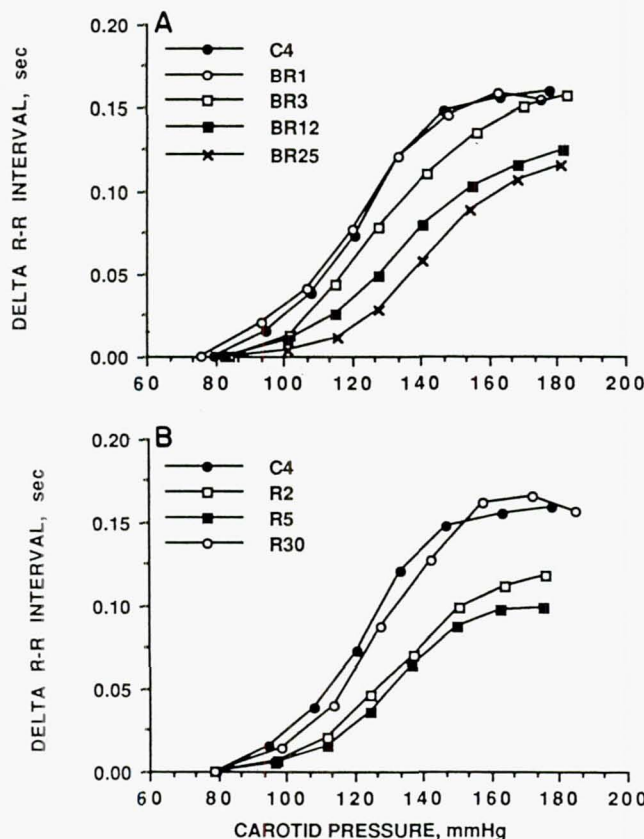


FIG. 2. Carotid baroreceptor-cardiac reflex response relationships. A: relationships generated on days 1, 3, 12, and 25 of bed rest (BR) and control day before bed rest (C4). B: relationships generated on days 2, 5, and 30 of ambulatory recovery after bed rest (R) and C4.

rest and immediately on the termination of the 30-day bed rest (R1). Posture tests before and after bed rest began with the subject lying supine for 60 min; this was followed with the subject sitting in bed with his feet hanging over the side of the bed not touching the floor for an additional 60 min. Immediately after the sitting test at the end of bed rest, subjects underwent a 5-min active stand test. The subjects were instructed to stand still with their feet placed 12 in. apart with their weight evenly distributed and to refrain from moving. Thus, contractions of the leg muscles were those required to

support stationary standing. Blood pressure and heart rate were measured at the end of minutes 3 and 5 of standing. Because heart rate is a physiologically important variable, we characterized cardiac responses to standing with heart rates rather than R-R intervals. In all cases, these heart rate values represented the peak heart rate achieved during standing. At the beginning of each test, a butterfly needle was inserted into an ante-cubital vein. Venous blood samples were drawn before sitting and at 5, 15, and 60 min after sitting in posture tests both before and after bed rest for the determination of norepinephrine and epinephrine. Blood samples were not drawn during standing.

Analysis of baroreflex responses. Previous studies using similar techniques have shown that intrasubject variability is so great that a meaningful fit to a four-parameter logistic equation is not possible for many subjects (13, 16). Therefore, baroreflex relationships were reduced to the following parameters for statistical comparisons: range of R-R interval responses, maximum and minimum R-R interval responses, maximum slope, position of operational point $[(\text{control R-R} - \text{minimum R-R})/\text{range}] \times 100\%$, and carotid distending pressures at minimum and maximum R-R intervals and at maximum slopes. Maximum slopes were determined by application of least-squares linear regression analysis to every set of three consecutive points on the response relationship to find the segment with the steepest slope. The carotid distending pressure at maximum slope was the point halfway between the pressures bracketing the maximum slope. These parameters are illustrated in Fig. 1B.

Statistics. Since baroreflex data for all subjects were found to be normally distributed with the Shapiro-Wilk normality test (28), parametric statistical analyses were used. A repeated-measures analysis of variance (ANOVA) technique with contrasts was carried out (25) to determine differences between measurements during the control and experimental sessions. Correlations were sought with least-squares regression (20). Syncopal and nonsyncopal groups were compared with Wilcoxon's rank sum test (1). A two-way ANOVA with days as the factor and subjects as the block was used to determine differences in resting levels of norepinephrine and epinephrine across days. Bonferroni's multiple comparisons

TABLE 1. Resting blood pressures, catecholamines, plasma volume, and characteristics of the carotid-cardiac baroreflex response before, during, and after bed rest

	C4	BR1	BR3	BR12	BR25	R2	R5	R30
SBP, mmHg	118±2	115±3	121±3	121±3	122±2	118±4	118±3	120±4
DBP, mmHg	75±3	76±2	73±2	73±2	72±2	78±3	74±2	77±2
MAP, mmHg	89±2	89±2	89±2	89±1	88±1	91±3	89±2	91±2
Base-line R-R interval, s	0.930±0.039	1.021±0.046*	0.894±0.032	0.882±0.032*	0.828±0.031*	0.802±0.033*	0.810±0.027*	0.862±0.021†
Min R-R interval, s	0.893±0.039	0.960±0.045*	0.845±0.031	0.833±0.027*	0.786±0.027*	0.748±0.029*	0.776±0.028*	0.791±0.031†
Max R-R interval, s	1.058±0.052	1.126±0.055*	1.006±0.051	0.963±0.039*	0.908±0.040*	0.870±0.037*	0.880±0.036*	0.958±0.024
R-R interval range, s	0.165±0.02	0.166±0.02	0.161±0.02	0.130±0.02†	0.122±0.02*	0.122±0.02*	0.104±0.02*	0.167±0.02
Max slope, ms/mmHg	3.65±0.64	3.47±0.57	3.25±0.52	2.52±0.35†	2.52±0.33*	2.28±0.34*	2.65±0.60*	3.09±0.52
Max slope, bpm/mmHg	-0.227±0.024	-0.223±0.029	-0.214±0.025	-0.168±0.019*	-0.161±0.025*	-0.188±0.025*	-0.170±0.023*	-0.229±0.039
CDP at max slope, mmHg	127±4	121±4	133±4	129±5	139±6	136±5	136±4	131±6
CDP at max R-R interval, mmHg	165±5	168±7	175±6	174±8	164±9	172±3	172±5	173±7
CDP at min R-R interval, mmHg	85±4	81±5	90±4	93±8	95±6	82±3	86±5	88±6
Norepinephrine, pg/ml	184±21	216±23		148±18	171±17	224±24		
Epinephrine, pg/ml	92±25	72±14		66±17	63±8	72±12		
Plasma volume, ml	3,676±163		3,237±82*	3,140±96*	3,108±98*	3,509±109‡		

Values are means ± SE. SBP and DBP, systolic and diastolic blood pressure; MAP and CDP, mean arterial and carotid distending pressure; C, BR, and R, before, during, and after bed rest, respectively. * $P < 0.05$ vs. C4 value; † $P \leq 0.06$ vs. C4 value. ‡ Calculated from hematocrit and hemoglobin values from C4 and R1.

were used when any factor differences occurred. A three-way ANOVA using test days (before and after bed rest), posture (supine and sitting), and subjects as factors was used to determine differences in the responses of norepinephrine and epinephrine during the posture tests. Subjects were used as a block to absorb any variability between the subjects. Dunnett's multiple comparison was used when any factor differences occurred.

RESULTS

Baroreflex responses. Average baroreflex response relationships for all subjects are depicted in Fig. 2 and are listed in Table 1. These relationships demonstrate that both maximum slopes and ranges of responses decreased progressively with continuing bed rest (Fig. 2A) and did not return to base-line values by the 5th day of recovery (Fig. 2B). The response relationship shifted significantly on the R-R interval axis but did not shift on the pressure axis (Table 1). There were parallel shifts in base-line, minimum, and maximum R-R intervals. All increased (slightly) after the initial 2 h of bed rest (BR1), progressively decreased through R2, and did not recover by R5. Resting systolic, diastolic, and mean arterial pressures, as well as the carotid distending pressures at minimum and maximum R-R intervals, and maximum slope did not change with bed rest. The position of the operational point was $21.6 \pm 8.0\%$ before bed rest and was not altered significantly by bed rest.

Catecholamine responses. Compared with C4, resting norepinephrine and epinephrine levels were not significantly altered during bed rest (Table 1). The elevation of norepinephrine levels induced by the change from supine to sitting positions was significant but did not differ between posture sitting tests before and after bed rest (Fig. 3). There was no change of epinephrine levels during the posture test either before or after bed rest.

Orthostatic responses. Complete data from the stand test were obtained for 10 subjects. Four subjects experienced syncope or presyncope, and the remaining six subjects tolerated the posture test with no noticeable difficulty. Although heart rate increased with standing

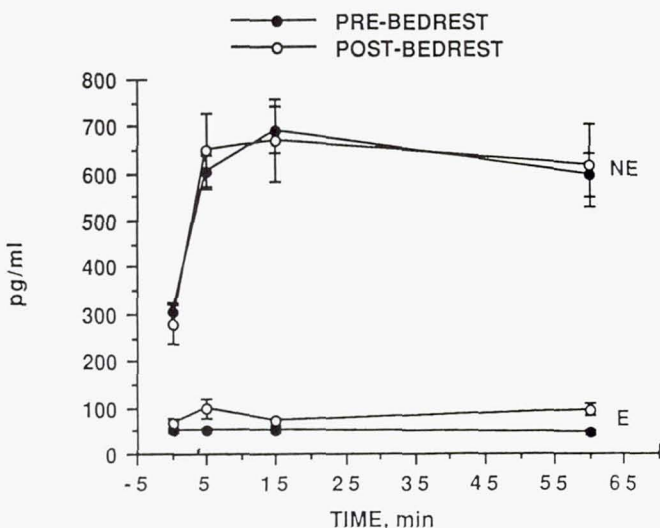


FIG. 3. Plasma concentrations of norepinephrine (NE) and epinephrine (E) during supine rest (time = 0 min) and at 5, 15, and 60 min of sitting before and after bed rest.

in both syncopal (23 ± 5 beats/min) and nonsyncopal ($+49 \pm 10$ beats/min) subjects, the increase was significantly less ($P < 0.05$) in the syncopal group (Fig. 4A). Nonsyncopal subjects maintained the same systolic pressure before and after bed rest, but syncopal subjects experienced significant reductions of systolic pressures (Fig. 4B). Average standing diastolic pressure after bed rest was increased relative to pressure before bed rest ($P < 0.05$), but there was no difference in this response between syncopal and nonsyncopal subjects.

Figure 5 demonstrates that the attenuation of the baroreflex response relationship was less in nonsyncopal (A) than in syncopal (B) subjects. The reduction in maximum slope of the response relationship from 4.0 to 2.2 ms/mmHg for the syncopal group was significantly greater ($P = 0.042$) than the reduction from 3.1 to 2.7 ms/mmHg in the nonsyncopal group (Fig. 6A). The change in the maximum slope of the baroreflex response relationship from C4 to BR25 correlated ($r = 0.70$, $P = 0.030$) with the change in systolic pressure from supine

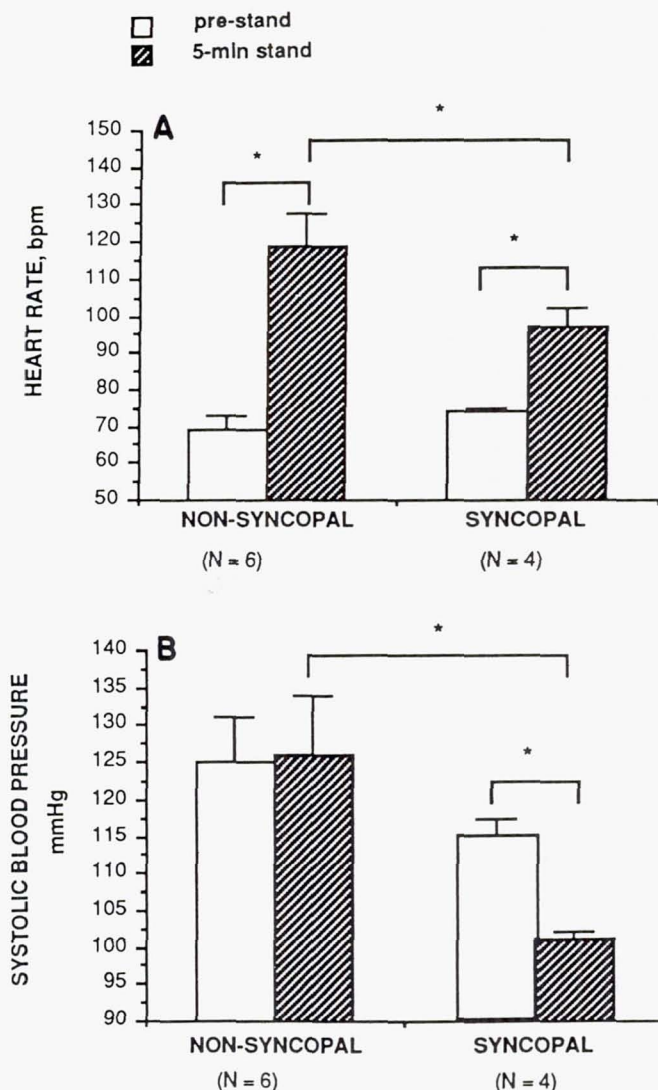


FIG. 4. Heart rate (A) and systolic blood pressure (B) during supine rest (pre-stand) and at 5 min of standing in nonsyncopal and syncopal subjects after bed rest. *Differences at $P < 0.05$.

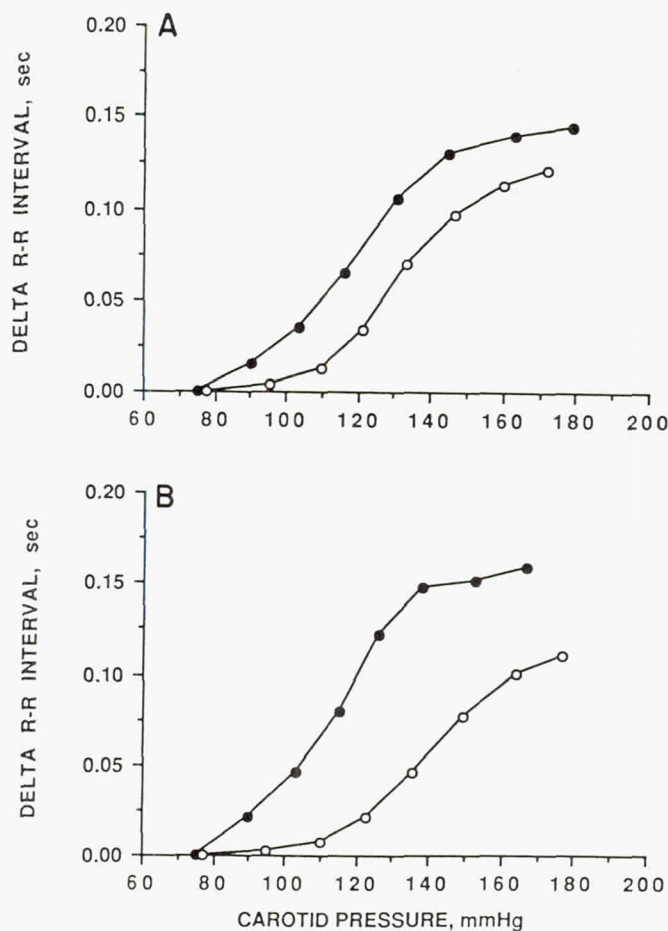


FIG. 5. Carotid baroreceptor-cardiac reflex before (●) and at the end (○) of bed rest in nonsyncopal (A) and syncopal (B) subjects.

to standing during the posture test before bed rest (Fig. 6B).

Plasma volume responses. Mean resting plasma volume for all subjects decreased by ~15% by BR3 but showed no further reduction through the end of bed rest (Table 1). An estimate of resting plasma volume calculated from hematocrit and hemoglobin levels at C4 and R1 indicated a rapid return to levels before bed rest by the 1st day of ambulation. Reductions ($P < 0.05$) of plasma volume, from 47.3 ± 2.6 ml/kg on C4 to 40.1 ± 1.7 on BR25 in nonsyncopal subjects, were not significantly different from those of syncopal subjects (whose average reduction of plasma volume, from 46.4 ± 1.5 to 42.6 ± 1.7 ml/kg, was actually less). There was no significant correlation between changes of plasma volume during bed rest and changes of baroreflex slopes ($r = -0.17$, $P = 0.376$).

DISCUSSION

We measured vagally mediated carotid baroreceptor-cardiac reflex responses in 11 healthy men before, during, and after 30 days of 6° head-down bed rest to test the hypothesis that baroreflex malfunction contributes to the orthostatic hypotension associated with this model of simulated microgravity. The major findings of this study are that head-down bed rest leads to substantial and progressive impairment of baroreflex function and that the development of baroreflex malfunction is related significantly to the occurrence of hypotension during

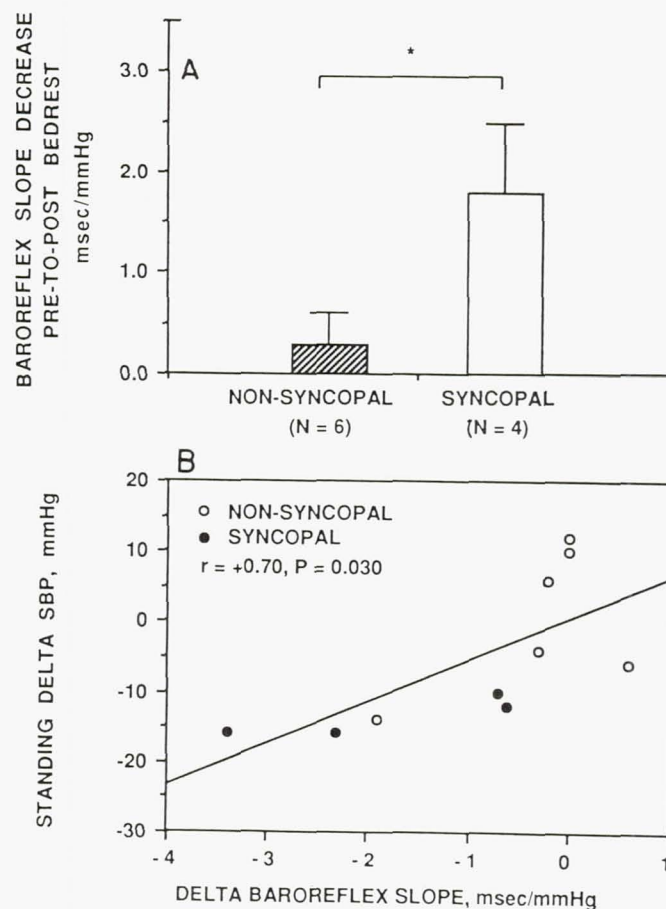


FIG. 6. Reduction in maximum slope of carotid baroreceptor-cardiac reflex relationship after bed rest in nonsyncopal and syncopal subjects (A) and relationship between change in maximum slope and change in systolic blood pressure (SBP) during stand test after bed rest (B). *Differences at $P < 0.05$.

standing after bed rest. Our data may be the first to demonstrate in healthy humans that impairment of baroreflex function can be provoked experimentally, is associated with clinical symptoms, and can be reversed completely.

Vagal responses. Sigmoidal baroreceptor-cardiac reflex relationships shifted on the R-R interval axis during bed rest (Table 1). Average base-line R-R intervals increased after 2 h of bed rest and then declined progressively during the bed rest period. Baroreflex changes became significant by BR12 and persisted through at least 5 days of ambulatory recovery. Some of our results confirm those of Billman et al. (2), who reported that primates experience reductions of R-R interval prolongation after phenylephrine injections, between days 7 and 28 of horizontal body casting.

We chose R-R intervals to express cardiac responses to carotid stimulation because of their linear relationships to vagal-cardiac nerve activity (17, 21). Rowell (22) demonstrated that elevations in base-line heart rate of 40–120 beats/min during exercise result in reduced baroreflex sensitivity when the slope is based on the R-R interval-blood pressure relationship; however, the baroreflex slope is unaltered when expressed by the heart rate-blood pressure relationship. This controversy raises an issue of whether the reduced maximum slope of the

baroreflex response in our bed rest subjects might have resulted from their increased resting base-line heart rates. We therefore calculated maximum slopes of the carotid-cardiac baroreflex using both measured R-R interval and calculated heart rate. Despite changes in base-line R-R intervals during bed rest, the reduction in maximum slope of the baroreflex relationship during bed rest was established using both expressions of cardiac responses (Table 1). Thus a change in resting heart rate of ≤ 10 beats/min in the present study did not have an effect on the calculated maximum slope of the stimulus-response relationship of the carotid-cardiac baroreflex.

Increases and reductions of gain and maximum and minimum R-R intervals during carotid baroreceptor stimulation tended to occur in parallel with elevations and reductions of base-line R-R intervals. This relationship raises the issue that increasing resting heart rate may alter the baroreflex response. Our data do not explain this relationship. However, compared with C4, the base-line R-R interval was significantly elevated on BR1 and R30 without significant changes in baroreflex slope. Furthermore, different studies that involved serial measurement of baroreflex responses in the same subjects during circadian changes in base-line heart rate (16) and over 10 wk of exercise training (9) have documented acute and chronic alterations in base-line R-R intervals without changes in reflex gain. It therefore appears unlikely that changes in base-line R-R interval could explain the reductions in baroreflex slope observed in our subjects.

Progressive baroreflex shifts were not associated with alterations of resting systolic, diastolic, or mean arterial pressures or the carotid distending pressures that elicited minimum and maximum R-R interval responses (near baroreceptor threshold and saturation). However, there was impairment of subjects' capacities to respond to and compensate for changes of blood pressure during standing. Our finding that impairment of vagally mediated baroreflex responses may be associated with impaired blood pressure regulation but unchanged resting blood pressure is not without precedent. Conway et al. (10) and Watson et al. (29) showed that, in hypertensive patients, impairment of vagally mediated baroreflex responses (abrupt R-R interval prolongations after blood pressure elevations provoked by bolus injections of phenylephrine) is associated with increased variability of arterial pressure. Cowley et al. (11) reported that, in conscious dogs, sinoaortic denervation (an extreme form of baroreflex impairment) is associated with supranormal blood pressure variability during upright posture. Our data are consistent with these earlier observations and support the idea that the primary function of baroreflexes may not be to set chronic levels of arterial blood pressure but to buffer transients in blood pressure, including those caused by postural changes.

Blood volume changes. One mechanism that may be responsible for our findings is impairment of baroreflex control by blood volume reductions. Harrison et al. (15) suggested that central blood volume changes alter carotid baroreflex responses, because head-down tilt reduces and head-up tilt increases R-R responses to neck pressure stimuli. In our study, the time courses of changes in

plasma volume and slope of the baroreflex relationship were not parallel. Plasma volume fell significantly by the 3rd day of bed rest, at a time when maximum slope of the baroreflex response relationship had not changed. After the initial 3 days of bed rest, plasma volume remained at constant low levels for the remainder of bed rest, but maximum baroreflex slopes declined progressively between days 12 and 25. Plasma volume was restored to control levels within the 1st day of ambulation after bed rest, but maximum baroreflex slopes remained depressed for at least 5 days after bed rest. Thus, although our data do not rule out a contribution from reduced blood volume, they point away from reductions of blood volume as the sole cause of the baroreflex abnormalities that developed during bed rest.

Sympathetic responses. Against expectations, there was no evidence for reciprocal impairment of sympathetic responses; catecholamine levels were comparable in lying and sitting positions before and after bed rest (Fig. 3). We used antecubital vein plasma norepinephrine levels as indexes of sympathetic nerve activity, because they are related closely to baroreflex-mediated changes of muscle sympathetic nerve activity (12). Although we measured catecholamines in lying and sitting positions, we did not measure catecholamine levels during standing. Burke et al. (5) found that muscle sympathetic nerve activity increased when subjects changed from lying supine to sitting and increased further when they stood. Thus our data on sympathetic mechanisms are limited and do not exclude an abnormality of reflex sympathetic neural control. Such an abnormality might be expected on the basis that both vasoconstriction and cardioacceleration (19) during prolonged standing result importantly from increased sympathetic outflow.

Orthostatic hypotension. A subgroup of four subjects became syncopal during 5 min of standing after bed rest. Compared with subjects who tolerated upright posture well after bed rest, syncopal subjects demonstrated an inability to increase heart rates adequately, despite a greater unloading of baroreceptors (i.e., a greater reduction of systolic blood pressure, see Fig. 4). Although other factors, such as reduced circulating blood volume (3, 6), increased leg vascular compliance (18), and reduced responses of vasoactive hormones (3, 6, 24), may have contributed to orthostatic intolerance, there were no significant differences in blood volume reductions and catecholamine responses between syncopal and nonsyncopal subjects. Significant elevations in average calf compliance from before and after bed rest, which have been reported for these subjects (8), did not differ between syncopal (4.0 ± 1.3 to 5.3 ± 0.9 vol%/mmHg) and nonsyncopal (4.0 ± 0.5 to 5.1 ± 0.7 vol%/mmHg) subjects.

Syncopal subjects demonstrated greater reductions in maximum slope and buffer capacity (range of R-R interval change) of their baroreflex response relationship than nonsyncopal subjects. The degree of impairment of baroreflex function after bed rest correlated directly with the greater reduction in systolic blood pressure (Fig. 5B) and the smaller tachycardia during standing. Our data may be related to those of Cowley et al. (11), who found that conscious dogs with sinoaortic baroreceptor denervation had smaller heart rate increases and greater blood

pressure reductions during upright posture than dogs with intact baroreflexes.

Although our results do not prove that baroreflex abnormalities cause orthostatic hypotension after head-down bed rest, they support such a notion and raise the intriguing possibility that baroreflex impairment contributes to the orthostatic hypotension experienced by astronauts after spaceflight. Most US astronauts experience symptoms of lightheadedness, and some progress to presyncope or syncope during standing after spaceflights of 7–10 days (3). We hypothesize that as the duration of spaceflight increases, progressive attenuation of baroreceptor-cardiac responses occurs and leads to greater postflight orthostatic intolerance. If this hypothesis is validated, development of effective countermeasures for postflight orthostatic hypotension following projected 90- to 180-day Space Station missions might include techniques to increase vagal-cardiac activity and baroreflex responsiveness before reentry.

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Exposure to Stressful Environments

Strategy of Adaptive Responses

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Abstract. Any new natural environment may generate a number of stresses (such as hypoxia, water lack, and heat exposure), each of which can produce strains in more than a single organ system. Every strain may in turn stimulate the body to adapt in multiple ways. Nevertheless, a general strategy of the various adaptive responses emerges when the challenges are divided into three groups. The first category includes conditions that affect the supply of essential molecules, while the second is made up by those stresses that prevent the body from regulating properly the output of waste products, such as CO₂ and heat. In both classes, there is a small number of responses, similar in principle, regardless of the specific situation. The third unit is created by environments that disrupt body transport systems. Problems may arise when there is a conflict between two stresses requiring conflicting adaptive changes. An alternative to adaptation, creation of micro-environment, is often favored by the animal.

Survival of a population often hinges on its ability to occupy new ground. Relocation may be forced upon a species by a variety of causes, chief among which are natural disasters, gradual changes in the climate, displacement from the ecological niche by a dominant competitor, or the disappearance of some other animals or of plants upon which the group under consideration depends in one respect or another, usually as a food source. Spill-over into an adjacent territory also occurs when successful colonization creates an unacceptable increase in population density. Unfortunately for the migrators, contiguous areas frequently have different climatic features and therefore provide new living conditions, making existence syno-

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Table I. Stresses produced by some environments

Environmental challenge	Stress				
	O ₂ balance	heat balance	water balance	food supply	etc.
Altitude	+++	++	++	?	
Diving	+++	+++			
Desert life		+++	+++	+++	
Jungle life		+++			
Confinement	++	++	++	?	
Acceleration	+++				
Etc.					

A number of environments are listed in the leftmost column. The *major* stresses created by each are shown in the subsequent columns, with a gradation indicated by the number of + signs. 'Etc.' indicates that the table should be extended in both directions by adding environments and stresses.

nymous with the talent to adjust to altered, and often unfriendly, environments. The gamut of possible adaptive responses is extensive, covering subtle modifications in behavioral patterns at one end of the spectrum and major remodeling of anatomical or biochemical structures at the other extreme, with functional modifications making up the mid-range. Some of the adaptive changes may occur during the lifetime of a single individual, others require several generations.

In discussing the fundamentals of adaptation, Dejours [1] defined this process as 'a change minimizing the physiological strain which results from a stressful environment'. The chain of events can therefore be summarized by the following progression:

New environment → stress → strain → adaptation.

The scientist who works at the bench is usually interested in a single cause-consequence pair, studying an isolated variable under carefully controlled and reproducible conditions, and therefore has no problem with this scheme. His colleague in the field who attempts to provide real-life synthesis of laboratory findings is in a far less favorable position because this deceptively simple sequence is complicated by the fact that each of the steps is multiplicative: an environment often creates more than one stress, each of

Table II. Strains caused by environmental stresses

Stress	Strain				
	circulatory system	respiratory system	urinary system	digestive system	etc.
O ₂ balance	+++	+++		+	
Heat balance	+++	+++			
Water balance	+++	+	+++	++	
Etc.					

Major strains produced by each stress are shown, with the gradation indicated by the number of + signs. 'Etc.' extends the table in both directions.

which – if severe enough – may generate several strains, and any one of these usually triggers numerous adaptive mechanisms. Table I describes the stresses that are caused by selected environments while table II lists the strains that correspond to some of these stresses.

Because the plethora of relationships shown in tables I and II would make a list of environments and adaptive steps both lengthy and unrewarding, it is necessary to resort to a simpler approach, and to attempt to sketch adaptation in broader lines, focusing on salient points only. For a more comprehensive, up-to-date review, see Senay [2].

The 'milieu extérieur' acts both as a source and as a sink, supplying the organism with its basic requirements such as metabolites, water and oxygen, and accepting in return carbon dioxide and other wastes, including heat. Thus, animals have to face two major types of environmental problems: on the 'input' side there can be a mismatch of supply to demand, while on the 'output' side, the surrounding medium may not be able to handle breakdown products, forcing the body to store them. Let us examine both sets of challenges in more detail.

Environments Causing 'Input' Problems

The common adaptive strategies that one can detect are those with which we are familiar in the field of economics; when availability fails to meet demand one can reduce consumption, switch to alternate sources or

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develop new routes of supply, rely on substitutes, improve internal transportation, and prioritize distribution so as to ensure that vital operations are maintained. If the problem is recurrent and can therefore be anticipated, one should stockpile essential materials before the emergency occurs. The way in which living organisms adopt these measures when they are subjected to stressful environments can be illustrated by what is intended to be a selection of appropriate examples rather than an exhaustive list.

Lack of Metabolites: Complete or Partial Starvation

Adaptation to a limited caloric intake triggers a number of the responses we have just reviewed. There is a decrease in metabolism, which has the obvious consequence of stretching the insufficient supplies. This drop in demand, reviewed by Grande [3] for man and animals can be attributed to two causes: (1) body mass shrinks, reducing the size of the body to be fed, and (2) the metabolic rate of active tissue slows down gradually, stabilizing after a few weeks. The first of these two changes cannot be considered to be adaptive; the second certainly is. In parallel, the organism develops alternate sources of essential materials, an example being provided by generation of glucose from other entities, in particular lipids. Storage of fat before an extended period of fasting has been well documented in hibernators and in birds preparing to migrate, and is discussed in terms of the incubating penguin by Le Maho [4] elsewhere in this volume.

Water Balance

Whereas some natural stresses (such as problems of O₂ supply and of insufficient food intake) can occur on their own, i.e. in the absence of other perturbing factors, this is seldom the case for water balance. Lack of water is usually associated with thermoregulatory problems, and occurs not only in very hot environments but also in extremely cold ones, where the energy needed to melt ice makes water in liquid form a scarce commodity. Furthermore, these two conditions may boost water loss, the first through sweating and/or increased insensitive water loss, the second mainly by decreasing the water vapor content of the inspired air.

Water economy can be achieved only by reducing outgo. Desert rodents produce feces which are practically devoid of water, urine becomes more concentrated, sweating is reduced. A number of steps can also be taken to diminish pulmonary water vapor loss, either by having it deposit in the upper airway during expiration and entraining it during inspiration, or simply by reducing minute volume. Clearly, a sizeable drop in ventilation is

tolerable only if it is coupled with a shift of the hemoglobin dissociation curve to the left, so as to maintain adequate oxygenation of the arterial blood.

Much has been made of the ability of the kangaroo rat to obtain water from metabolic sources, allowing the animal to live without intake of preformed water. It has been pointed out [5] that H_2O extraction from food is more complex than would appear and can be successful only under certain conditions: one can calculate that as the camel uses its fat reserves, it also obtains water but the increased O_2 requirement implies the need for extra ventilation and hence a rise in respiratory water loss. The latter may exceed the gain, leaving the animal with a water deficit!

It is well known that animals can carry water reserves. In addition, the behavior of some desert rodents generates an extracorporeal water store: by hoarding grain in their burrows, where it absorbs some of the moisture [5], they recycle part of the water they lose.

Oxygen Balance

Adaptation to an inadequate supply of oxygen deserves special mention for several reasons, the most important of which is probably that the body's O_2 stores are woefully small in comparison to its needs. While life without water or metabolites is possible for variable but extended periods of time, the total amount of oxygen stored in the body and available for emergency use can last only a few minutes at rest, much less at exercise.

Another unusual feature of this stress is that there is usually no shortage (or excess) in the *amount* of O_2 available, as is the case for water or food. Even at the top of Mount Everest, a climber would have an infinite number of oxygen molecules at his disposal; his problem is that this gas is presented at a low partial pressure, which hinders its transport.

Hypoxia

Decrease in partial pressure of inspired O_2 can be caused by one of two conditions: there may be either a drop in the fractional concentration of the gas in the environment, as occurs in burrows, or a reduction in total barometric pressure, as is found at altitude. The usual adaptive response is multifaceted, and some of its features depend on whether one is looking at a single acute episode, repetitive encounters with the stress, or chronic exposure. Just as is the case with starvation, a severe deficit in oxygenation leads to a reduction in consumption [6], but the strategy relies mainly on lowering the ventilatory and the circulatory resistances to oxygen transport so as to

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decrease the O_2 pressure drop at each step of the oxygen cascade. As a result of these changes, the total P_{O_2} difference between inspired and mean end-capillary blood – to the extent to which this is reflected by mixed venous blood – is reduced from more than 100 Torr at sea level to one-third of that value or less at high altitude. Note that whereas the ventilatory conductance can only be improved by increasing alveolar gas flow, the rise in circulatory conductance is due to both a quantitative factor, i.e. a boost in cardiac output, and a qualitative one, resulting from higher hemoglobin concentration and often a favorable displacement of the hemoglobin dissociation curve, this shift being particularly well illustrated in fish that breathe O_2 -poor water. When hypoxia is extreme or is combined with exercise, which increases the O_2 demand, another mechanism listed earlier, i.e. switch to a different metabolic pathway, is often invoked as the subject or animal satisfies part of the metabolic demand through anaerobic glycolysis. Clearly, this can take place for only a limited time, after which oxygen must be used to recycle lactate. For a more extensive discussion of the combined effects of hypoxia and exercise, see Cerretelli [7].

Some of the other general strategies which we have listed earlier are also implemented in specific cases of adaptation to low oxygen. As examples (1) a number of species have undergone evolutionary changes to survive in the presence of hypoxia: certain fish living in oxygen-poor water have developed lungs of various degrees of complexity, often using them as an auxiliary system, i.e. only when the water cannot supply the O_2 demand [8]; (2) diving mammals illustrate the benefits to be gained by prioritizing blood flow distribution and hence oxygen delivery: severe vasoconstriction of all the beds that subserve areas whose continuous unimpeded function is not imperative allows the animals to allocate the scarce oxygen reserves practically entirely to their heart and their brain; and (3) the blood volume of these diving mammals and their hemoglobin concentration are both high, increasing the amount of O_2 that can be stored prior to the dive [9].

Hyperoxia

At the cellular level, there are chemical reactions that scavenge superoxide and associated products, as reviewed by Crapo [10]. In contrast to the rich, assorted spectrum of systemic reactions to low oxygen, adaptive responses to hyperoxia appear to be limited to vasoconstriction and a moderate decrease in cardiac output, which reduces O_2 transport to the tissues, moderating the rise in cellular P_{O_2} . The input that triggers that

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Table III. Adaptive responses to 'input' environmental stresses

Response	Stress				
	lack of food	lack of water	hypoxia	hyperoxia	etc.
Decreased consumption	+++	+++	++		
Development of alternate sources/supply routes		++	++		
Substitution	++		+		
Transport optimization			++++	+	
Allocation of priorities	+	++	+++		
Stockpiling	++	+	++		

Only the most frequently used responses are shown. For details, see text.

response is not clear, although some experiments [11] indicate that the phenomenon is dependent, at least in part, on the P_{O_2} beyond the arterial tree.

One should note that, as opposed to hypoxia, which occurs in a variety of natural environments, hyperoxia is almost invariably an artificial challenge. It does occur in fish living in ponds or seawater pools where the photosynthesis of a rich aquatic flora creates diurnal P_{O_2} cycles with a peak that can reach 600 Torr [12], but not in air breathers. The existence of adaptive mechanisms, limited as they may be, is either fortuitous or the consequence of the fact that, as life's environment went from a reducing atmosphere to an oxidizing one, some responses against the increasing O_2 level may have emerged. Obviously, this explanation is tenable only for the cellular reactions, since complex organisms having a well-defined circulatory system developed long after oxygen had become part of our environment.

Overview of Responses

The parallelism of the various steps is emphasized in table III, which lists the most common features encountered in the environmental stresses we have just sketched. Because of space limitations, several challenges have been grouped under one heading. As an example, 'Hypoxia' covers such diverse conditions as the drop in P_{O_2} encountered at altitude or by fish living

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in waters with little oxygen, the decrease in oxygen fraction faced by the fossorial species, or the temporary lack of oxygen of an animal during a breathhold dive. Obviously, only some of the responses listed in one column will be elicited by one specific environment.

Environments Causing 'Output' Problems

With rare exceptions, problems on the 'input' side are caused by deficiency rather than by excess, since in the vast majority of cases an animal cannot be forced to avail itself of an oversupply but remains free to limit its intake to a desirable, safe amount. On the 'output' side, the stress may be either surfeit or scarcity: complete elimination of some of the important products of metabolism is often undesirable, and since many of these (such as heat and carbon dioxide output) have to be adjusted to maintain body levels within a narrow range, an abnormally high loss can be as close to catastrophic as reduced elimination.

Responses to environmental conditions that affect output are perhaps best understood in terms of a simple electrical model. In this analog, the material to be eliminated is compared to electrons moving from a high potential at the point where they are generated, to a low potential in the surrounding medium, through a series of resistors, each of which represents a step in the transport chain. The desirable level at each location – equivalent to voltage – is set by adjusting properly the different resistances. Environmental stresses may act on the system either by altering the potential of the sink or by affecting the resistance of the outermost transport link(s). It is easy to recognize immediately the possibility of three adaptive responses, namely (1) readjustment of the resistances in the transport chain; (2) change of the intensity of the current generated at the source; and (3) creation or enhancement of capacitances to better handle retention, in particular when this occurs in surges. We shall use stresses on the thermoregulatory system and on CO₂ elimination to illustrate these mechanisms.

Thermoregulation

Exposure to Heat

It is obvious that an increase in ambient temperature causes an immediate drop in the temperature gradient across the skin, which means that less heat can be dissipated if skin conductance remains fixed. The obvious

compensation, i.e., a decrease in the resistance to heat transfer across the outer insulation layers, is brought about mainly by vasodilation of the skin and the underlying tissues. At the same time, an attempt is made to cool the contact surface by evaporative heat loss, the efficiency of sweating depending of course on the ambient humidity. The problem is that these responses require an increase in peripheral perfusion, easily accomplished at rest, but not so at exercise, because (1) the skin competes with muscle for available blood flow, and (2) sweating decreases plasma volume and may therefore interfere with development of maximal cardiac output. Thus, core temperature rises during exercise, restoring (at least partly) the outward heat gradient. Interestingly, the acclimatized subject is presented with an additional problem: as he sweats more, he becomes dehydrated (and should compromise his maximum cardiac output) faster; some desert animals have risen to the challenge by developing mechanisms that allow them to maintain plasma volume while subjected to heat dehydration. For details, see the chapter by Horowitz and Samueloff [13].

Exposure to Cold

Cold stresses are generated not only when the body is exposed to a lower ambient temperature, but also when body heat is drained at too fast a rate because of an increase in thermal capacity or conductance of the surrounding medium. Not surprisingly, adaptation to cold invokes the same regulatory mechanisms as heat exposure, but in the opposite direction. When the stress is essentially continuous, as in the case of diving mammals, physical insulation is provided by a thick layer of subcutaneous fat. When the exposure is transient, but repeated, resistance to heat flow is increased by peripheral vasoconstriction, in particular in acclimatized individuals, whose maximum tissue insulation exceeds that of nonacclimatized subjects having the same cutaneous thickness [14]. Metabolism rises, generating more heat, and can of course be boosted dramatically by shivering [15].

Carbon Dioxide Retention

The first and foremost defense mechanism, increase of the CO_2 conductance, that is, hyperventilation, is brought into play whenever possible. Storage of the retained CO_2 is also attempted, and in this respect one must distinguish between animals that are faced continuously with high levels of CO_2 , such as rodents living in burrows, and those that are exposed only intermittently. In either case the problem is to maintain pH, but this is accomplished by different means. The animals that live in a high CO_2

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Table IV. Adaptive responses to 'output' environmental stresses

Response	Stress			
	heat	cold	CO ₂ retention	etc.
Resistances adjustment	+++	+++	+++	
Flux adjustment	+++	+++		
Increased capacitance			+	
For details, see text.				

environment retain bicarbonate in their plasma and extracellular fluid; on the other hand, diving mammals rely on better buffering power of their blood.

Overview

Table IV shows the similarity between the various mechanisms just listed. The reservations expressed in relation to table III apply here also.

Environment and Transport Problems

The preceding two sections have made it clear that optimization of the internal transport system is often the critical feature of adaptation. It is therefore appropriate to indicate here that certain environments leave the input and output sides unaltered but disrupt the ability of the body to either supply the cell or detoxify it. One such condition is exposure to high pressure, where the rise in respired gas density makes it difficult to maintain adequate alveolar ventilation. Another is increased acceleration, especially along the long axis of the body, that is foot-to-head. This stress, which is equivalent to an increase in gravitational pull, is always manmade, but is nonetheless interesting from both the scientific and the practical points of view. It acts primarily by magnifying the normal tendency of the blood to pool in the dependent parts of both the systemic and pulmonary circulations, thus reducing venous return and hence cardiac output. This sequence often lowers brain perfusion to dangerous levels or even reduces it to zero,

causing the dreaded 'black-out' of military pilots, who may be subjected to accelerations of more than 10 G.

Duration of exposure to high G is usually much too short to evoke adaptive mechanisms, but one must remember that the strain depends on the pressure exerted within the vessels in the dependent parts of the body, a pressure that is a function not only of gravity, but also of the height of the blood column. Therefore, very tall animals have to defend themselves against edema formation in the lower part of their limbs even at normal G. The complete picture of the adaptive mechanisms is not yet clear, but it is known that the skin of the giraffe's extremities is very tight and can therefore act as a natural anti-G suit.

The topic of acceleration fits perhaps best within the scope of this review when one considers the opposite end of the scale. i.e. microgravity, a situation in which the effects of gravity are counterbalanced to a greater or lesser extent. The best known example is that of astronauts in orbit, in whom centrifugal and gravitational accelerations are equal and opposite. Alternatively, during immersion, buoyancy compensates for gravity in the systemic circulation, while recumbency minimizes the length of the body axis on which gravity operates; these two types of challenge are often used as a 'poor man's zero G simulation', and have demonstrated that if the exposure is long enough, some of the adaptive mechanisms that have been developed in response to our normal 1G environment may be blunted or even disappear, a phenomenon known as deconditioning. The reasons and time course of this process are reviewed by Pendergast et al. [16] in this volume.

Adaptation: Problems and Alternatives

From what has been said so far, it is fairly clear that most environmental stresses can be minimized by the numerous adaptive responses that they trigger; we must balance the picture by indicating that the reactions themselves may not be free of problems. A conflict will arise when two different stresses, caused by the same environment, call for opposite responses. This divergence is sometimes obvious: a subject exposed to altitude must often respond to both hypoxia and water intake limitations, but whereas the former calls for hyperventilation, water conservation demands a decrease in respiratory water loss. At other times, a beneficial reaction fails to take place, and we are left with the speculation that it may be opposed by a stress and a counterstimulus that we do not see. Such is the case in the response to

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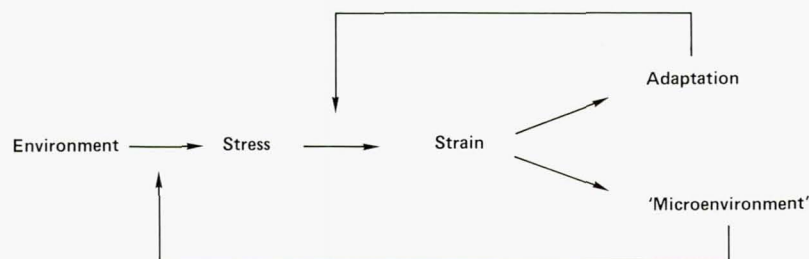


Fig. 1. Possible responses to new environments. As defined by Dejours [1], adaptive mechanisms minimize the strains caused by a stressful environment. The alternative, creation of a 'micro-environment', allows the animal to avoid or minimize some of these stresses. Such a strategy relies more on behavior than on physiological changes.

exposure to altitude, where, in spite of the increased pulmonary arterial pressure, distribution of ventilation/perfusion ratios is not improved. This has led Kreuzer et al. [17] to conclude: 'It is of great interest why such a potentially effective defense against hypoxia as a reduction in oxygen gradient should be excluded from the acclimatization process. There is no direct evidence to explain this, but in all probability the exclusion is obligatory as a result of other adaptive mechanisms.' Finally, at times, it is the adaptive response itself which creates an additional stress, as exemplified by dehydration occurring as a result of response to a heat load. Another important consideration is that long-range adaptive processes triggered by one environment may make it more difficult to respond to another stressful situation.

If adaptation to environmental stresses presents serious problems, is there an adequate substitute? Let us examine briefly two alternatives. The first is simply an increased tolerance to changes in physiological status. Along these lines, one can think of the Australian aborigine whose temperature drops during the night and of the camel who stores heat during the day and loses it during the cool desert night.

Many species have attacked the problem in a different fashion: by creating a micro-environment in which to live, one reduces or abolishes the stress and thus the need to minimize the strain (fig. 1). This micro-environment may be communal like the beaver's log house and man's climate-controlled housing, or individual like the eskimo's suit that provides its owner with semitropical conditions. Where at all possible, this strategy seems superior because it does not require changes in structure or in

physiological function and therefore ensures species survival without sacrificing flexibility. One should note, however, that in making their own 'milieu-demi-exterieur', animals often trade one stress for another. That they choose to do so raises the tantalizing question of hierarchy of stresses, a fruitful field for further investigation.

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Exercise Effects on the Size and Metabolic Properties of Soleus Fibers in Hindlimb-Suspended Rats

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GRAHAM SC, ROY RR, WEST SP, THOMASON D, BALDWIN KM. Exercise effects on the size and metabolic properties of soleus fibers in hindlimb-suspended rats. *Aviat. Space Environ. Med.* 1989; 60: 226-34.

The soleus atrophies rapidly when a rat is subjected to hindlimb suspension (HS), probably as a result of a decrease in the force encountered by the muscle. To test this premise, adult female rats were HS and half the rats were exercised (HS-EX) on a treadmill for 1.5 h · d⁻¹ at 20 m · min⁻¹ and a 30% grade. After 4 weeks, the midbelly of the soleus was prepared for histochemical analysis. Fibers were typed as dark or light staining for myosin ATPase, alkaline preincubation. Fiber size and quantitative histochemical enzyme activities of succinate dehydrogenase (SDH) were determined using a computer enhanced image processing system. In comparison to age-matched controls, the soleus wet weight was 69 and 30% smaller in HS and HS-EX rats. The mean cross sectional area of the dark ATPase fibers was reduced by 46 and 18% and light ATPase fibers by 69 and 48% in the HS and HS-EX, respectively. The percent dark ATPase fibers increased from 10% in the control rats to 19 and 17% in the HS and HS-EX. In both suspended groups, SDH activities in light ATPase fibers were 40% higher than control. The SDH activity of the dark ATPase fibers of HS-EX was 20% higher than control, while the dark ATPase fibers of HS were similar to control. To determine the degree to which these increases in SDH could be related to reductions in fiber size rather than increases in the actual amount of protein, integrated activity (activity/min x area) was calculated per fiber. The integrated activity of SDH suggests a net loss of this enzyme in the light and dark ATPase fibers of HS, but only in the light ATPase fibers of the HS-EX rats. In summary, daily treadmill exercise ameliorated, but did not prevent, the muscle fiber atrophy induced by HS in a slow extensor muscle. Further, SDH activity per fiber was maintained or elevated in the HS and HS-EX rats regardless of the variable changes in fiber size induced by suspension and endurance type exercise.

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IT IS APPARENT that the forces encountered during everyday situations are critical in the maintenance of normal skeletal muscle properties, especially for extensor muscles. When these forces are no longer present, the affected muscles exhibit characteristic changes. Generally, the muscles atrophy and demonstrate shifts in their mechanical properties towards those found in "faster" muscles. This has been shown to be true in several models which are assumed to induce dramatic decreases in *in vivo* muscle force production, such as spinal isolation (26), spinal transection (2,27), limb immobilization (34), hindlimb suspension (9,18,31,35), and spaceflight (10,15,19). It also appears that not all muscles are affected equally by the removal of these ground reactive forces. For example, the antigravity muscles of the lower leg, the extensors (e.g., the soleus and medial gastrocnemius) are affected to a greater degree than are the flexors (e.g., the tibialis anterior and extensor digitorum longus) (9,12,22,23,32,35). It also has been shown that within a functional group of muscles, the "slower" muscles, i.e., those muscles which have a relatively high proportion of fibers that stain lightly for myosin ATPase, alkaline preincubation, are more responsive to the removal of force than muscles which have a relatively high proportion of darkly staining fibers (24,35). In this light, the soleus, a predominantly slow extensor muscle, appears to be a good model to study the effects of weightlessness or unloading on skeletal muscle.

Given the apparent importance of muscle loading in maintaining normal muscle properties, particularly in the extensors, it is likely that exercise can be used to attenuate the functional decrements associated with chronic decreases in muscle force. For example, Roy *et al.* (27) have shown that assisted treadmill walking for 30 min · d⁻¹ results in a significant reduction in the atrophy and associated decrement in force capabilities of the soleus muscle resulting from chronic, low thoracic spinal cord transection in cats. Similarly, several recent, mainly preliminary, reports (12,22,30,33) have

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suggested that treadmill exercise of various durations and intensities may help to ameliorate the atrophic response associated with hindlimb suspension (HS). Other forms of loading, such as grid climbing with attached weights (13) and chronic stretch of the hindlimb muscles (18) in HS rats also have been shown to benefit the affected muscles. Thus, the purposes of the present study were to 1) evaluate the morphologic and succinate dehydrogenase (SDH) adaptations occurring in soleus fibers identified by myosin ATPase type when unloaded for a period of 4 weeks, and 2) assess the effectiveness of an endurance exercise program in ameliorating these changes.

METHODS

Experimental design and suspension procedures: Adult female Sprague-Dawley rats (approximately 275 g, body weight, 8 weeks of age) were obtained from Taconic Farms, Germantown, NY. The rats were assigned randomly to one of three groups: sedentary control (CON, $n = 8$), hindlimb-suspended (HS, $n = 8$) or hindlimb-suspended plus exercise (HS-EX, $n = 8$). Rats from each group were housed in identical cages and maintained in a light and temperature controlled environment. Food and water were provided *ad libitum*. Both HS groups were subjected to a suspension period of 28 d. The suspension model used has been described in detail (32,33). Briefly, the rats were suspended by the application of a tail cast traction bandage covering less than one-half the tail surface and thus allowing adequate thermoregulation through the tail. The tail cast was attached to a swivel hook mounted at the top of the cage, allowing free 360° rotation. The height of each animal was adjusted to allow the rat to support its weight and to move about freely on its forelimbs while the hindlimbs were elevated to prevent contact with the floor or the sides of the cage. Animals were checked daily for signs of tail lesions or discoloring, unusual breathing patterns, or undue discomfort. An animal showing any of these signs was immediately removed from the study.

Rats in the HS-EX group were exercised on a treadmill with their tail casts attached to the treadmill cover rails to prevent them from dragging. Initially, the rats were run for 10 min daily with 5 min added each day until they were running $1 \text{ h} \cdot \text{d}^{-1}$. Subsequently, the running period was increased by $10 \text{ min} \cdot \text{d}^{-1}$ until a final bout of $1.5 \text{ h} \cdot \text{d}^{-1}$ was attained. The exercise was performed at a speed of $20 \text{ m} \cdot \text{min}^{-1}$ and at a 30% grade.

Tissue preparation: Following 28 d of HS, the rats were anesthetized with sodium pentobarbital ($50 \text{ mg} \cdot \text{kg}^{-1}$, i.p.) and the soleus, along with other muscles, and the adrenal glands were removed. The tissues were rinsed with cold saline, trimmed of excess fat and connective tissue, blotted dry, and wet weighed. Subsequently, the rats were sacrificed by exsanguination. A 5–10 mm thick cross section was taken from the mid-belly of the soleus, mounted on cork in a manner such that the fibers were perpendicular to the cork surface. The sections were quick-frozen in isopentane cooled to -160°C by liquid nitrogen. This process was completed within 15 min after removal from the animal.

The tissue was cut in $10\text{-}\mu\text{m}$ thick serial sections in a cryostat maintained at a temperature of -20°C and mounted on coverslips. Sections then were stained qualitatively for myosin adenosine triphosphatase (myosin ATPase, alkaline preincubation, $\text{pH} = 8.8$, and acidic preincubation, $\text{pH} = 4.35$) as described by Nwoye *et al.* (21). Although the assumption is generally made that fast-twitch fibers stain darkly and slow-twitch fibers stain lightly at an alkaline preincubation, this is unlikely to be true in a strict sense because in many cases there is not a clear separation of the physiological properties into two populations of motor unit types. However, this staining property does provide a consistent means of separating fibers according to the sensitivity of myosin ATPase activity to pH. Quantitative histochemical methods to determine SDH activities have been published recently (11,12,20,24). Briefly, the activity of SDH was determined in a medium containing 100 mM phosphate buffer ($\text{pH} 7.6$), $10 \mu\text{M}$ sodium azide, $20 \mu\text{M}$ 1-methoxyphenazine methylsulfate, 1.5 mM nitro blue tetrazolium, 5 mM ethylenediaminetetraacetic acid (disodium salt) and 48 mM succinate (disodium salt). The reaction was run at room temperature in the dark, and stopped at 8 min with repeated washings of distilled water. All sections were air dried, mounted with Aq mount and stored on an image processing system within 6 h. The quantitative histochemical assay for SDH has been found to be linear up to 12 min (20). The reactions were terminated prior to this point, thus the optical density per min readings (see below) represented a steady-state enzymatic reaction.

Tissue analysis: One representative sample of fibers from each muscle was chosen for analysis. In order to remain consistent, a region located at a particular site (i.e., based on anatomical features) in the cross section and containing a mixture of fiber types was selected. Four pairs of alternating sections were cut for the quantitative SDH staining reactions. One section in each pair was incubated in a medium either containing or lacking substrate. Sections incubated without substrate acted as tissue blanks to correct for nonspecific staining. The final optical density for each fiber was equal to the mean value obtained from sections with substrate minus the value of the tissue blanks lacking substrate. The quantitative histochemical processes have been verified biochemically with respect to tissue thickness and time of incubation (20). In addition, only a 3.3% coefficient of variation was observed in the protein analyses of 15 serial sections cut at a $10\text{-}\mu\text{m}$ thickness. These data indicate that the optical density readings are directly related to the volume of muscle tissue in the sections.

Tissue sections were digitized on a computer enhanced image processing system and stored on magnetic tape as described previously (11,12,20,24). Briefly, this system is composed of a light microscope (Zeiss M14), an image-digitizing television system (Eye Com II, Spatial Data System model 108PT) with a high-speed arithmetic unit, a line printer (Printronix), a magnetic tape drive (Kennedy model 9110) and a minicomputer (PDP-11/34) with dual-platter disc drive. An array of picture elements (640×480 pixels) are quantified to 256 grey levels that are then converted automatically to optical density. An operator-controlled joystick allows

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objects to be defined and analyzed independently. The camera is calibrated for light intensity with monochromatic light-interference filters. The camera's dark current is taken to be equivalent to 0% transmission of light and its saturation point is at 100% transmission. The optical density range is from 0.002 to 2.0. All light with a wavelength <450 nm in each density measurement is eliminated with a cut-off filter.

Approximately 25-60 adjacent fibers were analyzed from each section. These fibers were typed as darkly or lightly staining for myosin ATPase based on their optical density. Subsequently, fiber cross-sectional area (CSA) was determined and the reaction products from the SDH histochemical reaction were quantified. Interrelationships between fiber size and enzyme activities were determined from these samples. Care was taken to select fibers which appeared to be cut perpendicular to their longitudinal axis. Also, fibers that had freezing artifact or appeared to be degenerative were not analyzed quantitatively. The percentage of dark and light ATPase fibers of each muscle was determined by qualitatively typing between 1,600-2,400 fibers in each muscle.

Statistical procedures: A procedure was used which allowed for an unequal number of observations. Significant differences ($p < 0.05$) between groups were determined using a nested one-way analysis of variance. The Bonferroni (Dunn) t -test was used for determining significant differences between the three groups and consisted of performing three unpaired t -tests using the separate estimate of common variances and an adjusted significance level of $p < 0.017$ (i.e., $0.05/3$).

RESULTS

Body, muscle, and adrenal weights: Following 28 d of HS, the mean body weight of the HS group was significantly smaller than that of the CON group (Table I). In contrast, the HS-EX mean body weight was similar to CON and significantly larger than HS. Soleus wet weights were approximately 70 and 30% smaller in HS and HS-EX when compared to CON. Adrenal weights were not significantly increased due to the suspension protocol but were over 50% larger in the HS-EX in comparison to CON. These data suggest that the exercise regime, in combination with the suspension, was stressful for the rats.

Fiber type and size: The muscles from CON rats had

TABLE I. BODY, MUSCLE, AND ADRENAL WEIGHTS, AND PERCENT DARK ATPASE FIBERS IN CONTROL (CON), 28-D HINDLIMB-SUSPENDED (HS), AND 28-D HINDLIMB-SUSPENDED PLUS EXERCISE (HS-EX) RATS.

	CON (8)	HS (8)	HS-EX (8)
Body Weight (g)	295 ± 6	265 ± 7*	285 ± 7 [†]
Soleus Weight (mg)	107 ± 5	33 ± 3*	75 ± 4* [†]
Adrenal Weight (mg)	79 ± 5	83 ± 10	120 ± 4* [†]
Dark ATPase Fibers (%)	10 ± 2	19 ± 2	17 ± 4

Values are means ± standard error of the means. The number of rats in each group is given in parentheses. *Denotes a significant difference between CON and HS or HS-EX. [†]Denotes a significant difference between HS and HS-EX.

10 ± 2% fibers staining darkly for myosin ATPase at an alkaline preincubation (Table I). Suspension resulted in almost a doubling in the number of darkly staining fibers (i.e., from 10 to 19%), although the large inter-animal variability resulted in no statistically significant difference between the two group means. The HS-EX group had a percentage of darkly staining fibers that was between the CON and HS values.

In the CON group, the light ATPase fibers were 42% larger than the dark ATPase fibers (Fig. 1), a relationship consistent with previous data (11,12). The CSA of the light ATPase fibers in both suspended groups were significantly smaller than CON, i.e., 69 and 48% in HS and HS-EX, respectively. In contrast, the dark ATPase fibers atrophied by 46% in the HS, but only 18% in the HS-EX rats. As a consequence of this differential atrophy, the two fiber types became similar in size following 28 days of HS (Fig. 1).

The frequency distributions of CSA for both fiber types are illustrated in Fig. 2. In the CON group, the range of CSA in the light ATPase fibers is considerably greater than in the dark ATPase fibers (Fig. 2) and the mean CSA of the light ATPase fibers is approximately 50% higher than that of the dark ATPase fibers (Fig. 1). In the HS group, however, both the range and mean CSA are almost identical for the two fiber types. This observation emphasizes the greater relative and absolute atrophy of the light in comparison to the dark ATPase fibers following suspension (Fig. 1). The daily exercise regime partly prevented the shift towards smaller fiber sizes, particularly in the dark ATPase fibers where some fibers actually may have hypertrophied (Fig. 2).

Single fiber enzyme activities: Following 28 d of HS, the mean fiber SDH activity of the light ATPase fibers was 40% higher compared to CON ($p > 0.05$), while that

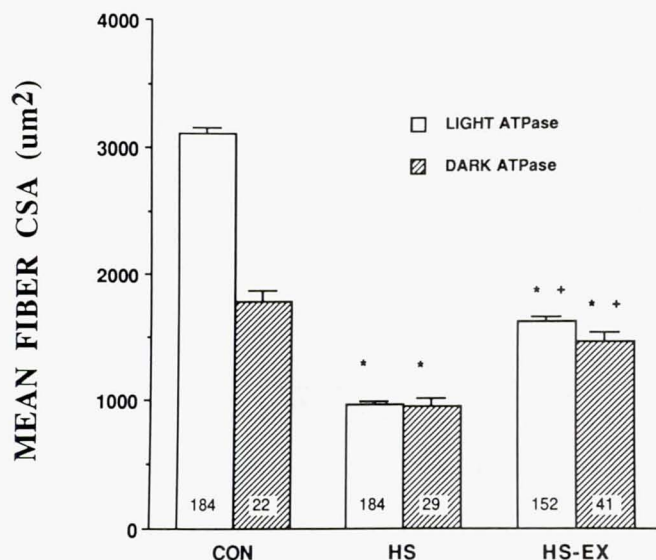


Fig. 1. Mean fiber cross-sectional area (CSA, μm^2) of light and dark ATPase fibers of the soleus of control (CON), and 28-d hindlimb suspended (HS) and HS plus exercise (HS-EX) rats. Number of fibers is indicated within each bar. Vertical bars are standard errors of the mean. *Significant difference between CON and either HS or HS-EX. [†]Significant difference between HS and HS-EX. $p = 0.05$.

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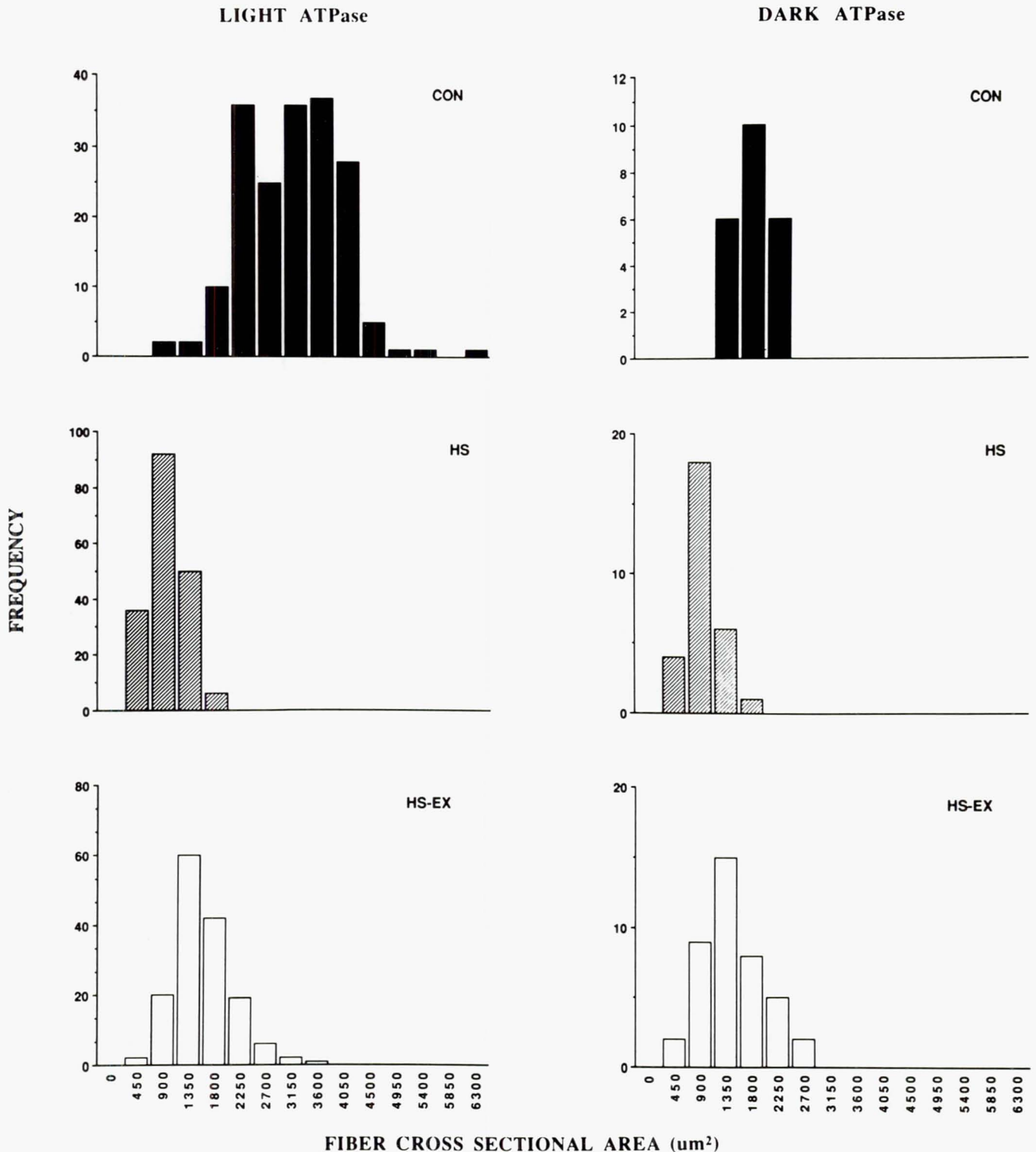


Fig. 2. Frequency distributions of fiber cross-sectional areas for light and dark ATPase fibers of the soleus of control (CON), and 28-d hindlimb suspended (HS) and HS plus exercise (HS-EX) rats.

of the dark ATPase fibers was similar to CON (Table II). Exercise potentiated the SDH activity in the dark, but not the light, ATPase fibers. The light ATPase fibers had a smaller range in SDH activities than the dark ATPase fibers in the CON group (Fig. 3). In all three groups, the light ATPase fibers had a smaller minimum SDH activity level than the dark ATPase fibers in their

respective groups. In both fiber types and in both HS groups, there was an increase in the proportion of fibers having SDH activities in the upper range of values (Fig. 3). However, the range of values at the lower end of the distribution was unaffected. Thus, it appears that only some of the fibers were affected by suspension. Overall, in each group, the dark ATPase fibers had a higher ($p >$

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TABLE II. SUCCINATE DEHYDROGENASE (SDH) ACTIVITY AND INTEGRATED SDH ACTIVITY (ISDH) OF SOLEUS MUSCLE FIBERS IN CONTROL (CON), 28-D HINDLIMB SUSPENDED (HS), AND 28-D HINDLIMB SUSPENDED PLUS EXERCISE (HS-EX) RATS.

	CON	HS	HS-EX
SDH (OD/min $\times 10^{-4}$)			
Light ATPase Fibers	42.77 \pm 1.28 (164)	59.97 \pm 2.56 (184)	59.76 \pm 2.35 (152)
Dark ATPase Fibers	70.80 \pm 6.11 (20)	78.83 \pm 5.34 (29)	84.66 \pm 5.02 (41)
ISDH [SDH activity (OD/min) \times CSA (μm^2)]			
Light ATPase Fibers	12.870 \pm 0.419 (164)	5.673 \pm 0.246 (184)	9.463 \pm 0.399 (152)
Dark ATPase Fibers	12.500 \pm 1.256 (20)	7.774 \pm 0.766 (29)	12.817 \pm 1.039 (41)

Values are means \pm standard error of the means, based on 4, 6, and 5 rats in the CON, HS, and HS-EX, respectively. The number of fibers analyzed for each assay is given in parentheses.

0.05) mean SDH activity than the light ATPase fibers; i.e., 40, 24, and 29% higher in the CON, HS, and HS-EX groups, respectively (Table II).

The total number of enzyme units in a fiber—i.e., the enzyme activity normalized by the area of the fiber (integrated activity (I) = enzyme activity (OD/min) \times CSA (μm^2))—was determined to account, in part, for the differential changes in CSA accompanying the suspension and exercise procedures. In comparison to CON, mean ISDH values decreased by 56 and 38% ($p > 0.05$) in light and dark ATPase fibers of HS rats, respectively (Table II). In comparison to the HS group, treadmill exercise increased the total number of enzyme units by approximately 65% ($p > 0.05$) in both fiber types. Further, the mean ISDH in the dark ATPase fibers was only slightly higher than CON (Table II), while the mean SDH activity was 40% higher. In HS rats, there was a general shift towards lower values for both fiber types (Fig. 4). In the HS-EX rats, the range of ISDH values for both fiber types was similar to CON. However, in the light ATPase fibers of HS-EX rats, there was a higher proportion of fibers in the lower range of values, while in the dark ATPase fibers a higher proportion of fibers were in the upper range of values (Fig. 4).

DISCUSSION

Body, muscle, and adrenal weights: The soleus mass was decreased to $\sim 30\%$ of CON after 28 d of HS (Table I). These data are consistent with the results of previous HS studies that report a dramatic loss in muscle mass in a relatively short period of time using a variety of suspension procedures and time intervals. For example, the magnitude of loss of soleus mass appears to be greatest within the first week followed by a slower, progressive loss over months; i.e., 7 (5), 20 (5), 30–45 (5,32), 40–50 (6,17,31,32), 40–70 (11,12,13,17,22,31,32,35, present data) and 55% (17) decreases in soleus mass after 3, 5, 7, 14, 28 and 90 days, respectively. Spaceflight also results in a rapid decrement in soleus mass. For example, Grindeland *et al.* (10) reported a 24 and 36% decrease in the soleus mass of large and small rats, respectively, after 7 d of spaceflight, while Ilyina-Kakueva *et al.* (15) reported a 32% decrease after a 20-d Cosmos flight.

The decrease in muscle weight associated with HS could be due, in part, to a decrease in the total body growth rate. Some studies have reported a decreased

growth rate of suspended rats based on body weight (7,17,28), whereas others have not (1,11,12,24,31,32, 33). Although the reasons for these discrepancies are unknown, one plausible explanation could be related to the amount of stress placed on the rats by the method of suspension. In the present study, the average adrenal weights were similar in the CON and HS group (Table I), suggesting a minimal level of stress imposed by our suspension procedures. In addition, the mean body weight of the HS group was only 10% ($p < 0.05$) lower than CON (Table I). Thus, the relative muscle weight (i.e., the muscle weight/body weight ratio) would be decreased in proportion to the absolute muscle weight, indicating that the atrophy is occurring primarily in the muscle. Similar conclusions have been drawn by Flynn *et al.* (7) who report decreases in the soleus wet weight/body weight ratios following HS. Together, these results suggest that the smaller muscle weights in HS rats are not entirely due to a lower body weight.

Other factors to consider in the interpretation of the smaller muscle masses associated with HS are the rates of protein synthesis and degradation. Jaspers and Tischler (16) report a slower rate of protein synthesis and an increased rate of protein degradation in the soleus following HS. Increases in the excretion of 3-methylhistidine and urea and in protease activity (31), markers of protein catabolism, have been shown after HS. Steffen and Musacchia (29) report a lower absolute RNA level in the soleus of HS rats, suggesting a reduction in the rate of protein synthesis. We did not measure protein metabolic rates in the present study. However, histological examinations of the muscle cross sections revealed a number of indicators of fiber degeneration; e.g., a small percentage of fibers were irregularly shaped and/or ragged, had central vacuolations, stained abnormally intense for the metabolic enzymes, etc. (data not shown). These histological abnormalities have been observed and discussed previously by other investigators following both HS (31) and spaceflight (23). No fibers showing these abnormalities were included in our quantitative data analyses.

Fiber size and type: The reduction in cross-sectional area of the light ATPase fibers paralleled the loss in muscle mass (i.e., $\sim 70\%$), while the reduction in the size of the dark ATPase fibers was smaller (i.e., $\sim 45\%$). However, most of the atrophy occurred in the light ATPase fibers since this type of fiber makes up the largest proportion ($\sim 90\%$ in the control rats) of the mus-

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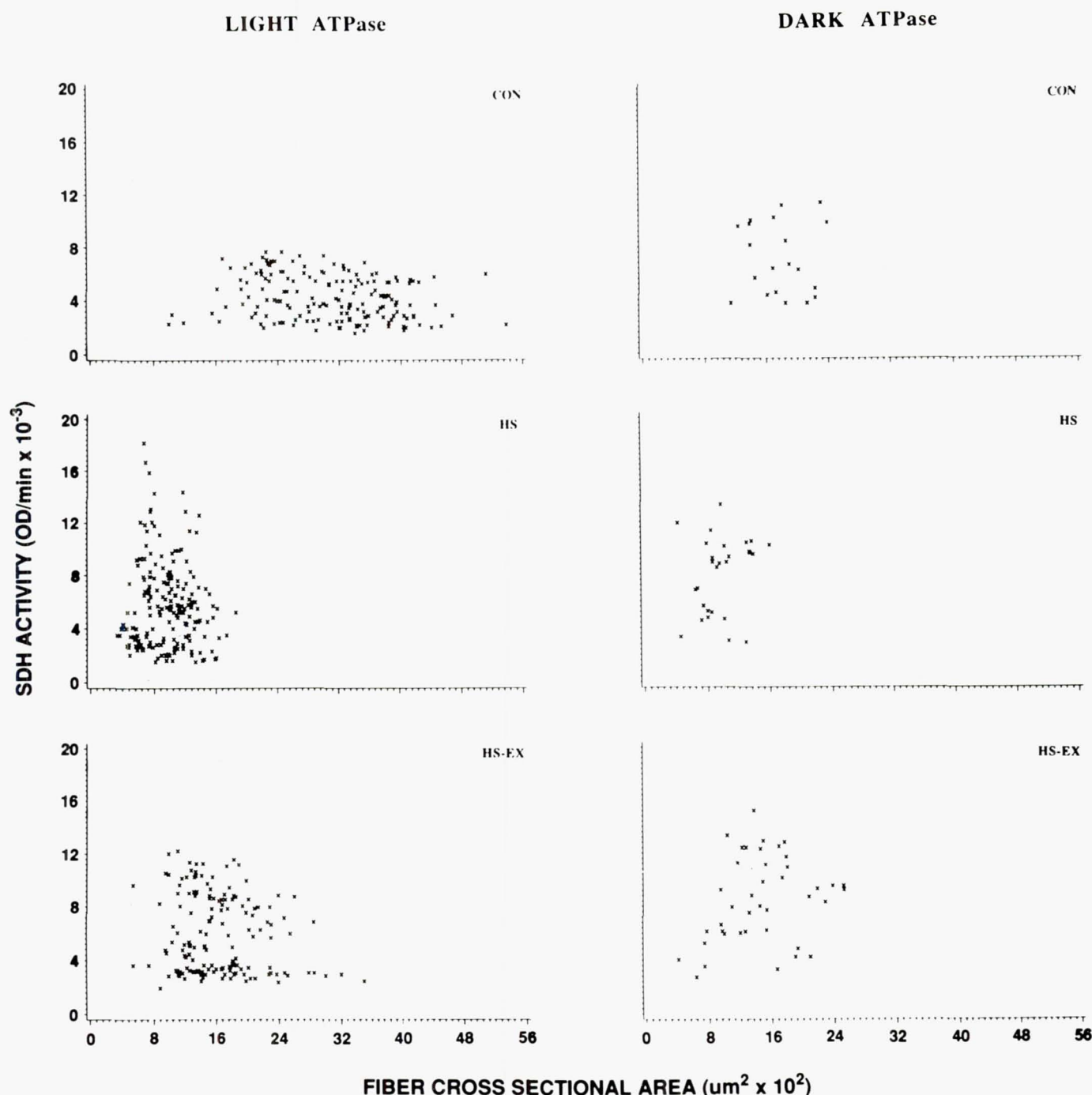


Fig. 3. Relationship between succinate dehydrogenase (SDH) activity and fiber cross-sectional areas for light and dark ATPase fibers of the soleus of control (CON), 28-d hindlimb suspended (HS), and HS plus exercise (HS-EX) rats.

cle. Differential atrophy in the two fiber types has been reported previously. In general, it appears that the larger, light ATPase fibers of the soleus (11,12,31), as well as the relatively small light ATPase fibers in mixed fast muscles (24), are more susceptible to the removal of their load-bearing function than the dark ATPase fibers. It is of interest also that the size of the fibers tends to reduce to a similar size suggesting that there may be some minimum size that is optimal for an unloaded muscle. This trend has been observed in several studies (11,31).

The percent dark ATPase fibers doubled in the HS

rats in comparison to CON, a finding consistent with previous histochemical (11,12,28,31) and biochemical (32,33) reports. These adaptations in myosin ATPase profile have been linked to the increases in the contractile speed properties of HS soleus muscles (6,31,35).

Single fiber SDH activities: SDH activity was 40% higher in HS rats compared to CON in the light ATPase fibers and was relatively unchanged (i.e., an 11% difference) in the dark ATPase fibers (Table II). Using the same quantitative histochemical procedures described in the present study, Hauschka *et al.* (11) observed a higher SDH activity of light ATPase fibers after 28 d of

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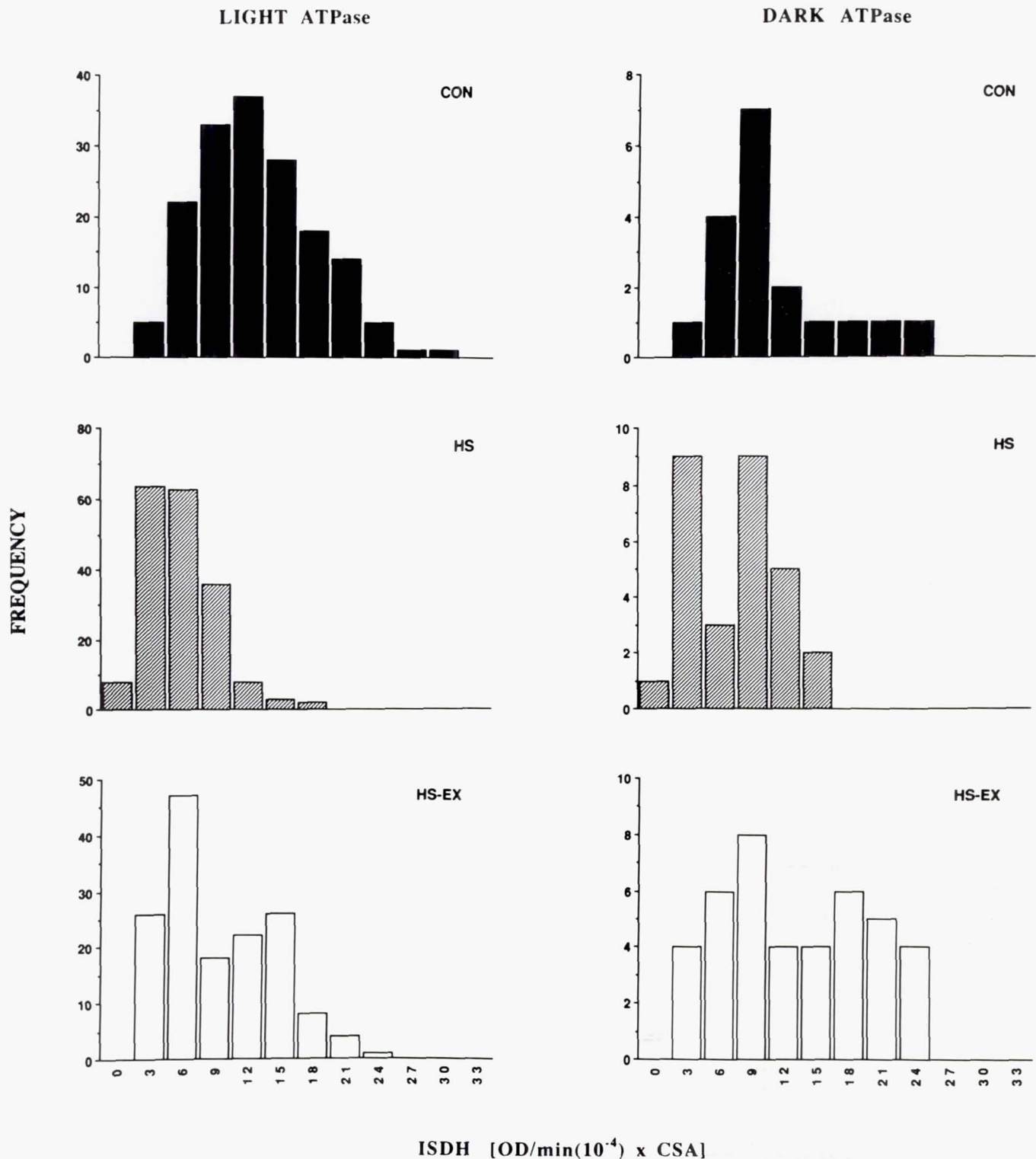
ISDH [OD/min(10^{-4}) \times CSA]

Fig. 4. Frequency distributions of integrated succinate dehydrogenase (ISDH) activity (activity/min \times CSA) for light and dark ATPase fibers of the soleus of control (CON), and 28-d hindlimb suspended (HS) and HS plus exercise (HS-EX) rats.

suspension, and Martin *et al.* (19) showed a maintenance of SDH activity in comparison to ground-based CON rats in both fiber types following a 7-d spaceflight. In contrast, there have been reports that the oxidative capacity of the soleus muscle is decreased following HS (4,7,28) and spaceflight (15,23). However, these latter reports are based on whole muscle homogenates, qual-

itative histochemical techniques and electron microscopy. These discrepancies may be due, at least in part, to the differences between the analysis procedures. For example, whole muscle homogenates include noncontractile tissues in the muscle mass, e.g., connective tissue. Based on previous data (7) and unpublished observations in our animals (A. Vailas, personal commu-

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nication), it appears that there is an increased concentration of connective tissue in HS rats. The presence of these non-muscle tissues would result in a dilution of the enzyme activities in whole muscle homogenates. The technique used in our analysis avoids this potential problem by quantifying the amount of reaction product formed during a given unit of time within the defined borders of a fiber.

In the present study the fiber enzyme activity was assessed as a function of fiber size. If a fiber atrophied without any concomitant absolute decrease in the number of enzyme units, then the enzyme activity would increase in proportion to the decrease in fiber size. To estimate the extent that this interactive effect may have occurred in this study, integrated optical density (I) values were calculated by multiplying the SDH activity times the area of the muscle fiber. This value indicates the relative number of enzyme units per fiber, assuming all SDH enzyme units have the same specific activity. Since the ISDH activity per fiber decreased in the HS group (Table II), there would have been a net loss of the SDH enzyme per fiber assuming no change in specific activity.

Since the SDH activity of the fiber is, at the least, maintained and in some cases increased after HS, it might be expected that the fibers would maintain their high resistance to fatigue. In fact, a maintenance of fatigue resistance of the soleus has been reported following 7 d (7,13,22) and 28 d (35) of HS. Similarly, fatigue resistance in the soleus of cats is maintained following prolonged periods of reduced neuromuscular activity; e.g., 6–12 months after spinal cord transection (2) or spinal isolation (26).

Effects of endurance exercise: Treadmill running was used in an effort to ameliorate the atrophic and metabolic changes associated with HS. The HS-EX group did exhibit several improvements over the HS group (Tables I and II). Both body weight and soleus wet weight were significantly higher in the HS-EX than the HS group, suggesting that exercise ameliorated, but did not prevent, the atrophic response to unloading. Similarly, Sullenger *et al.* (30) recently reported that 15 or 120 min of treadmill running at $13 \text{ m} \cdot \text{min}^{-1}$ and 0% grade for 5 d per week resulted in only a 32% decrease in soleus mass after 28 d of HS, as compared to a 49% decrease in the unexercised group. While these data suggest that periodic loading of the soleus during hindlimb suspension decreases the magnitude of the atrophy, they also show that similar effects can be obtained from a wide range of loading durations, e.g., 15–120 min.

The exercise protocol used in the present study, in conjunction with HS, apparently was very stressful to the rats, since the adrenal weights of the HS-EX group were 52% higher than either the CON or HS groups (Table I). It is known that glucocorticoids released under stressful conditions act directly on skeletal muscle, enhancing protein catabolism (8,25). In spite of this added stress, the soleus muscles of the HS-EX group were 130% larger than the HS group, suggesting that the treadmill exercise was a potent stimulus for maintaining muscle mass. In comparison to the HS rats, the mean cross-sectional area of both fiber types in the HS-EX group were closer to CON values, with the dark ATPase

fibers being only 18% smaller than CON (Fig. 1). These data suggest that the dark ATPase fibers were more responsive to this form of exercise.

The treadmill exercise protocol used has been shown to induce significant increases in the oxidative capacity of the involved skeletal musculature in normal rats (14). In the present study, the exercise ameliorated several of the metabolic-associated changes resulting from HS. The soleus fibers of HS-EX rats had elevated SDH activity compared to HS rats (Table II). Further, the number of enzyme units per fiber in the HS-EX was similar to CON in the dark ATPase fibers, and only 26% less than CON in the light fibers. Similarly, the ISDH in the HS-EX was about 65% higher than HS in both light and dark ATPase fibers.

In summary, it appears that the daily treadmill exercise ameliorated the atrophic response in the light and dark ATPase fibers of the hindlimb suspended rat soleus. Cross sectional areas of the light ATPase fibers were reduced to a greater degree than the dark ATPase fibers during hindlimb suspension, while the exercise appeared to prevent atrophy to a greater extent in the dark ATPase fibers. SDH activity was enhanced somewhat by the suspension protocol in both fiber types, with a greater increase in the light ATPase fibers. Daily exercise resulted in a further increase in the SDH activity of only the dark ATPase fibers. In addition, the increase in SDH activity generally resulted from a relatively greater reduction in cross sectional area than in SDH enzyme units as reflected by the changes in ISDH values.

ACKNOWLEDGMENTS

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Academy Transactions Note

PULMONARY FUNCTION IN MICROGRAVITY: SPACELAB 4 AND BEYOND†

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Abstract—The paper refers principally to the composition gradient of gases within the lung in various conditions of gravity, as revealed by exhaled breath. A rapid gas analyzer-based system has been developed for tests in Spacelab 4. The test sequence and expected results are presented.

1. INTRODUCTION

The large apico-basal gradients in alveolar size, ventilation, capillary distension and blood flow that exist in the erect human lung cause readily measurable gradients in regional lung function[1]. There are also gradients in the ratio of airflow (\dot{V}_A) and bloodflow (\dot{Q}_c), and thus in gas exchange and local alveolar gas concentration. Gradients in gas concentration also occur when a foreign gas is inhaled, because the ventilation per unit volume differs topographically[2].

These gradients in gas concentration can be seen during single, slow vital capacity exhalations, when we use a rapidly responding gas analyzer to sample gas concentrations at the lips. The heart causes pulsatile flow from the dependent lung regions, and thus there are "cardiogenic" oscillations in CO_2 and O_2 concentration due to the topographic gradients in gas exchange. Similarly there are oscillations of N_2 (the resident gas) if a breath of a diluent foreign gas has just been inhaled[3]. The lungs static mechanical properties cause the dependent regions to stop emptying first during exhalation, and this can be seen as a terminal inflection in gas concentration as flow from the upper regions predominates[4].

We can, therefore, study gravitational gas concentration gradients quickly and non-invasively. Michels and West[5] of our group did this in a NASA LearJet, which was flown through 30 second Keplerian arcs. Cardiogenic oscillations and terminal inflections in gas concentration virtually disappeared at zero-g confirming that the topographic gradients are gravitationally induced (Fig. 1).

We will soon be repeating the LearJet tests in Spacelab 4 (SL-4), along with a more extensive

package of tests to not only study influences of topographic gradients in lung function, but also to study the overall function of the lung in the presence of the headward redistribution of blood and liquid that occurs at the onset of exposure to microgravity. We will perform the test battery repeatedly preflight and postflight in both the supine and erect posture and at intervals throughout the SL-4 mission to study adaptation to the space environment, and the consequences of that adaptation postflight.

We describe here the details of the SL-4 experiment, and some of the more interesting predictions that we can make about the results we will obtain.

2. METHODS

2.1. Hardware

Single breath testing is almost ideally suited to the Spacelab environment. A large amount of information can be obtained by analysis of instantaneous gas concentration and flow at the lips, as the subject switches from breathing cabin air, to the inhalation and exhalation of a series of test gas mixtures[6].

The primary requirement is for a rapidly responding gas analyzer. NASA life sciences provide a Gas Analyzer Mass Spectrometer (GAMS) as a shared instrument. We use it to measure O_2 , CO_2 , N_2 , AR, C^{18}O and N_2O . It samples from the mouthpiece of our equipment at 60 ml/min and has a 10–90% response time of approx. 100 ms.

We have developed a rack mounted system, composed of a "bag-in-box" assembly, an electronics control assembly and a gas cylinder assembly (Fig. 2). The bag-in-box assembly allows the subject to be switched from breathing cabin air, to a series of premixed gases in separate breathing bags. These are enclosed in a rigid box, which is open to the cabin via a flowmeter (Fleisch No. 2 pneumotachograph) that maintains a continuous recording of respiratory flow throughout the test sequence.

The bag-in-box assembly is supported by the electronics controlling assembly (ECA). this allows inter-

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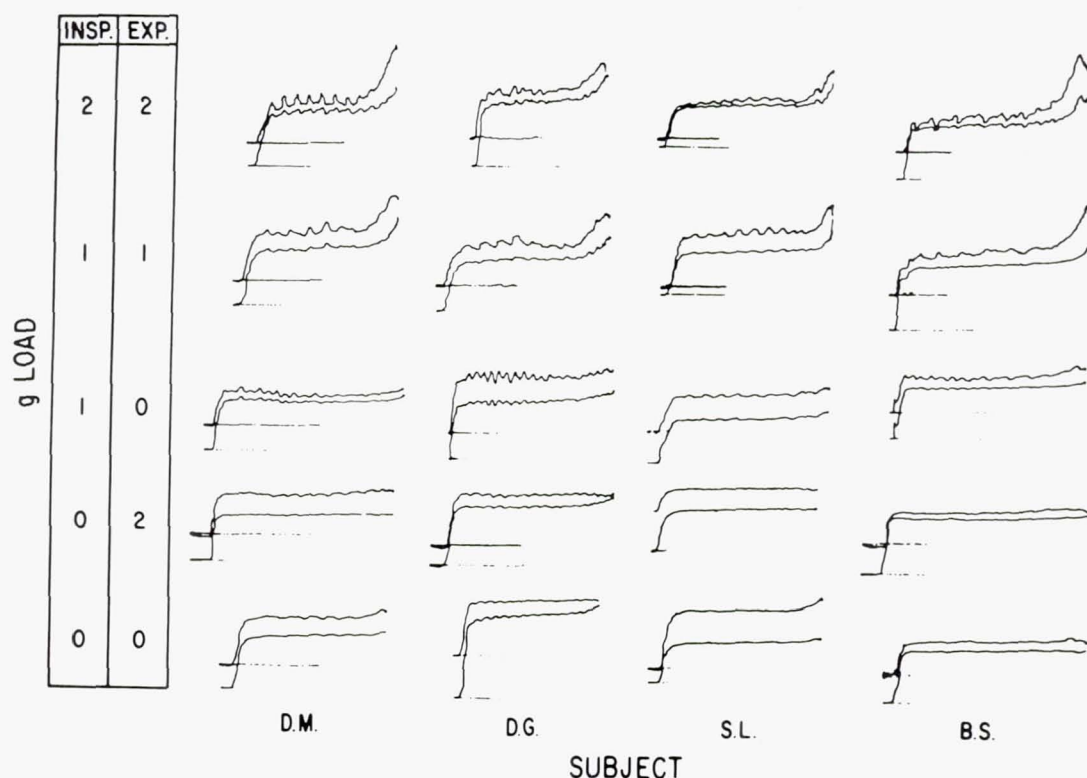


Fig. 1. Comparison of single breath washouts from four subjects (columns) at differing inspiratory and expiratory g loads (rows). Each subject inhaled a 150 ml bolus of argon, followed by pure O_2 to vital capacity just prior to the exhalations. Argon concentrations (upper traces) and nitrogen concentrations (lower traces) are plotted. Note the cardiogenic oscillations and terminal concentration rises: they are increased at 2- g , and markedly reduced at 0- g . From Michels and West[5].

action between the subject and a dedicated NASA microcomputer via an alphanumeric display and keypad. The ECA transmits analog data to the microcomputer, which in turn sends 14 channels of 12 bit A/D logged at 160 Hz, into the Spacelab data system. The system controls the subject's tidal volume, breathhold times and flowrates, by activation of solenoid valves. Tidal volume control requires precise linearization of the flowmeter using a 6809 microprocessor lookup table[7]. This limits drift of the generated volume signal to 20 ml during a 20 breath nitrogen washout.

The bag-in-box system is also interfaced to the gas cylinder assembly which dispenses the premixed gases (Table 1), and to the spacecraft vacuum system which enables bag emptying on computer command.

2.2. The test sequence

Syringe calibration of the integrating flowmeter is followed by automated sampling of all stored gas mixtures and the internal GAMS calibration gases. This is followed by a fixed test sequence that has been developed to minimize interaction and maximize efficiency[6] and is now finalized as in Table 1.

A period of quiet breathing on the mouthpiece is recorded allowing analysis of resting ventilation and gas exchange. This is followed by a standard single

breath nitrogen (SBN) washout, in which the subject inhales a 150 ml argon bolus at the start of a vital capacity breath of oxygen, then immediately exhales through a newly developed flow regulator at 0.5 l/s.

The subject then performs a hyperventilation-breathhold maneuver to maximize any gradients in the lung due to perfusion inhomogeneity, and then exhales at 0.5 l/s[5]. A standard single breath CO diffusing capacity ($D_L CO$) measurement follows[8] and is in turn followed by a period of oxygen breathing (multibreath nitrogen washout), in which the tidal volume and lung volume are rigidly controlled by solenoid valves[9]. A second $D_L CO$ is then performed with the hyperoxic $C^{18}O$ mixture, allowing calculation of the pulmonary capillary blood volume (V_c)[8].

A period of quiet cabin air breathing follows. Data collected during this period are used to define the parameters of a single compartment model of gas exchange. A vital capacity 0.5 l/s exhalation is then recorded, allowing analysis of intrabreath gradients in the respiratory exchange ratio (R), and thus of gradients in gas exchange, with a correction for continuing gas exchange using the model[6].

Rebreathing of the N_2O containing mixture follows. Data are analyzed to obtain cardiac output and lung volumes[10,11]. Finally, repeated forced ex-

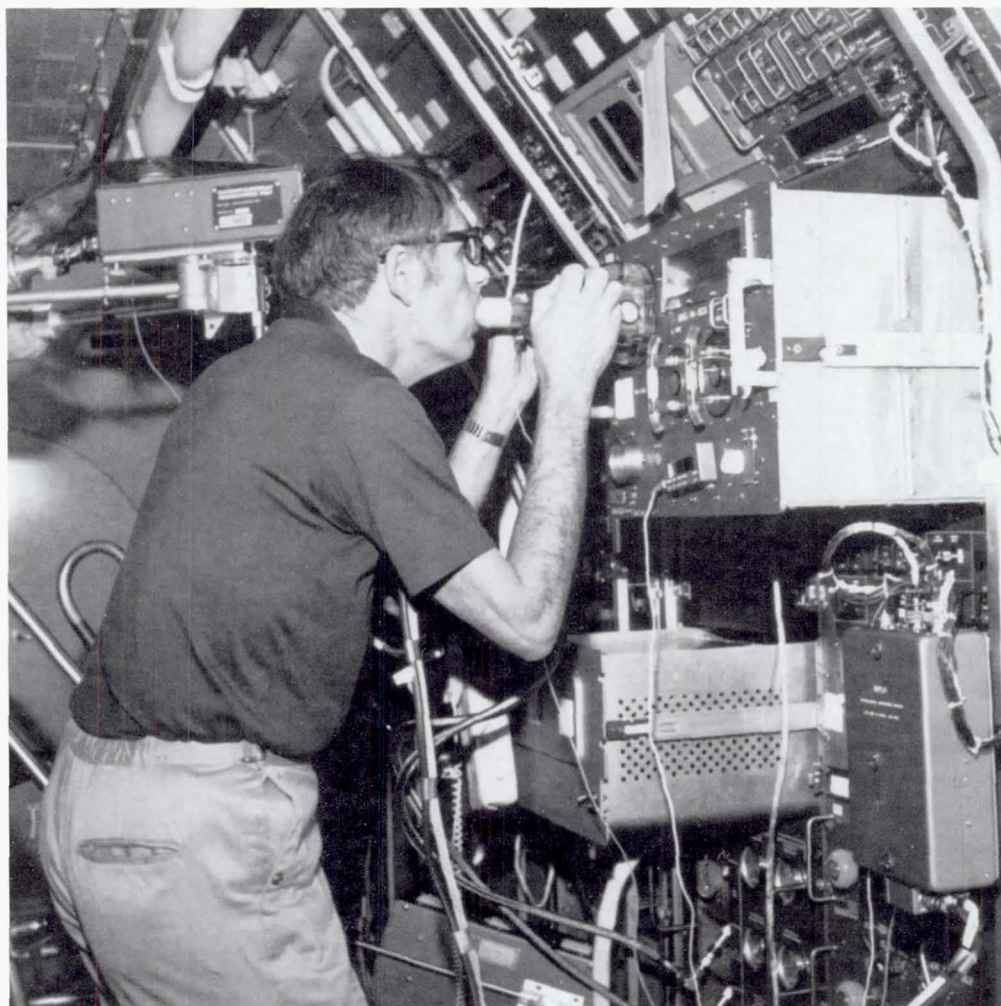


Fig. 2. Robert W. Phillips D.V.M., Ph.D., SL-4 payload specialist, using the rack-mounted experiment hardware. He is following prompts displayed on the electronics control assembly (ECA) above the deployed bag-in-box assembly. A mouthpiece probe is connected to the mass spectrometer below the bag-in-box.

piratory spirometry is performed. Data will be analyzed to define any changes from the characteristic 1-g flow/volume plot that is seen in each subject, in several gravitational orientations[12].

3. PREDICTIONS AND HYPOTHESES

3.1. Uneven ventilation

We expect the cardiogenic oscillations and terminal rises in the SBN argon and nitrogen traces to virtually disappear. Any residual oscillations or terminal inflections will be of great interest (in the LearJet experiment they were less significant because the lung had been deformed by accelerations immediately before entry into ballistic flight). Small cardiogenic oscillations may also persist because of lobar differences in specific ventilation. The terminal nitrogen rise may persist in part. It can be shown to be due to a different mechanism in abnormal subjects[13],

that is non-gravitational[14] and in the gravity free environment of Spacelab, a similar small non-gravitational signal may well emerge in normals.

The LearJet experiment showed that the SBN alveolar plateau slope persisted at 0-g although gravitational inhomogeneity was long thought to be its major cause. It is now thought to be largely due to intra-acinar diffusive/convective inhomogeneity, a consequence of asymmetry of branching at the alveolar duct level[15]. In the gravity-free environment the small gravitational component may become definable.

Analysis of the multibreath nitrogen (MBN) test will be particularly interesting. It has recently been shown that the normalized alveolar slope progressively increases from breath to breath. The rapid increase in slope in the first few breaths is a measure of the intra-acinar diffusive inhomogeneity, as evidenced by He/SF₆ studies and modeling[16]. The

Table 1

Test	Abbrev.	Gas mixtures	Reference
Resting gas exchange	RGE	Air	
Single breath N ₂ washout	SBN	(a) Oxygen (b) 79% Argon 21% O ₂	[5]
Distribution of perfusion	QDT	Air	[5]
Single breath diffusion test	DCO	0.3% C ¹⁸ O 10% Ar 21% O ₂ Balance N ₂	[18]
20 breath N ₂ washout	MBN	Oxygen	[9]
Followed by: hyperoxic diffusion (V_c) test		0.3% C ¹⁸ O 10% Ar Balance O ₂	[8]
\dot{V}_A/\dot{Q}_c test	VQD	Air	[6]
Rebreathing cardiac output	REB	2% N ₂ O 10% Ar 21% O ₂ Balance N ₂	[10] [11]
Spirometry	SPI	Air	[12]

increasing slope beyond the fifth breath depends on convective inhomogeneity at a grosser level. We can predict that the initial rate of change will persist on orbit. The subsequent rate of change will give new insights into the relative contributions of gravitational and non-gravitational mechanisms to convective inhomogeneity in the lung.

We can predict that the distribution of specific ventilation[9] obtained from the MBN data at 0-g, will be tighter than at 1-g. We can also predict that the SBN data and MBN data will be re-analyzed in new ways for many years, and will remain a valuable resource.

3.2. Perfusion

As in the LearJet experiment, we expect to see a big reduction in oscillations of O₂ and CO₂ concentrations. The Intrabreath R tracing should also show greatly reduced oscillations. The terminal rise in R seen in normal subjects, differs from the terminal fall, seen in subjects with airflow limitation[6]. It may be that a small fall in R, beyond that explicable by continuing gas exchange, is seen at 0-g. This would be evidence of the presence of a population of poorly ventilated alveoli with maintained perfusion; early evidence of lung "aging" that cannot be obtained in the presence of gravity. The persistence of small cardiogenic oscillations might suggest residual lobar perfusion differences.

Postflight, in the erect position, there may be very large gravitational gradients in blood flow. These would have some operational significance, as one could predict that gradients during re-entry would also be large[17]. We do not yet know if adaptation to 0-g causes an increase in post exposure gravitational gradients in the lung.

3.3. Pulmonary capillary volume and blood flow

We can predict that the changes seen in immersion[18] and bedrest[19] will occur. The cardiac output, D_L CO and its V_c component should increase on orbit. The extent of the increase and its persistence is not currently predictable, but it seems likely that the bedrest model is more realistic than the earlier immersion model, and changes will not persist after cardiovascular/renal adaptation. Postflight erect/supine differences may well be larger than those seen preflight.

3.4. Forced expiratory spirometry

When plots of instantaneous flow are made on an expired volume plot during maximum exhalation, individual subjects have a characteristic curve that has definite shape, and usually, very definite "bumps" that are reproducible. This characteristic "MEFV" curve differs as posture is changed[12]. It is thought that maximum flow is determined by wave speed of deflection of bronchial walls in a critical flow limiting segment. This segment moves from the trachea to the peripheral airways as lung volume diminishes. The supine MEFV curve shows significantly greater flow at large lung volumes, and interestingly, decreased flow at low lung volumes. It was predicted from current theory that gravitational inhomogeneity of airway pressure/area relationships should cause the opposite change at low lung volumes[12]. The decreased flow at low lung volumes, in the supine position, might be due to vascular/liquid cuffs around the small airways. If this also occurs at 0-g and especially if it returns toward normal with time on orbit, the vascular/liquid cuff theory will be supported.

4. CONCLUSIONS

For many years gravitational gradients in lung function have been recognized and studied. These gradients have made it difficult to study non-gravitational inhomogeneity in normal subjects. The development of a microgravity pulmonary laboratory, therefore, should provide a major resource for those who study the normal lung, both in the immediate and more distant future.

There are many practical applications that will also be of interest. While experience suggests that congestion of the lung with blood does not pose a problem at rest, there is less certainty that it is not a problem under severe exercise conditions, soon after the onset of weightlessness. There is also the possibility that inhalation lung injury (from aerosols, gases, and vapors) can occur in a closed 0-g environment. Aerosol deposition in the lung itself at 0-g, warrants study as sedimentation will no longer occur. Measured differences in deposition will not only be of practical, but also of great theoretical interest. High oxygen atmospheres that are required for pre-EVA

denitrogenation can exacerbate the effects of any pre-existing pathology on gas-exchange[20]. Total body denitrogenation itself is simply an extended "MBN test" and can be quantitated and compared with denitrogenation on the ground, from which it should differ because of the redistribution of bloodflow in the tissues of the body. We expect many groups to share this exciting initial exploration of pulmonary function in microgravity.

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Heart-Lung Interactions in Aerospace Medicine

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La Jolla, CaliforniaPlus ça change, plus c'est la meme chose.
Alphonse Karr, 1849

I. Introduction

Few of the heart-lung interactions that are discussed in this volume have been studied in any detail in the aerospace environment, but it seems to us that many such interactions must occur in the setting of altered accelerative loadings and pressure breathing. That few investigations are in progress suggests that clinical and academic laboratory investigators and aerospace organizations are further apart than during the pioneering work on pressure breathing and acceleration tolerance in the 1940s.

Our purpose is to reintroduce some of the perennial problems of aviation physiology as well as some newer aerospace concerns that may be of interest. We speculate about many possible heart-lung interactions, by necessity often drawing on data from within the aviation field, collected before the modern understanding of these interactions developed, or on recent laboratory data that may not be strictly applicable. In the field of zero-gravity effects, speculation inevitably outruns the sparse available data.

In this chapter, we assume less familiarity with the aerospace environment than with heart-lung interactions.

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II. Acceleration and Gravitation

The force exerted by the gravity of the Earth on a mass, m , is given by

$$W = m \cdot g$$

where W is the weight of the mass on the Earth's surface and g is the acceleration due to the Earth's gravity ($9.81 \text{ m} \cdot \text{sec}^{-2}$). Similar "inertial" forces are generated by acceleration (a), where

$$F = m \cdot a$$

The inertial and gravitational force vectors can be summed to give the total accelerative loading. In aviation, these loadings can be large, and it is convenient to compare these with the normal gravitational loading. This is done by normalizing a resultant acceleration (a) with reference to the standard gravitational acceleration g , leading to the 'G' system in which

$$G = a/g$$

In aviation, aerodynamic forces and thrust produce the accelerations. If the resultant of these forces (F) is twice the weight of a body, the accelerative loading is $2G$, and generally:

$$G = F/W = ma/mg$$

where W is the weight of the vehicle. An aircraft flying level at constant velocity, generates a net resultant aerodynamic force (here simply lift) equal to its own weight, and G is, therefore, 1. When speed is adequate, the lift force can be increased very readily by increasing the angle of attack of the wing (pulling on the yoke). This increases the lift/weight ratio (F/W), and thus G .

The mechanics of flight are such that an increase in lift (which is at right angles to the velocity vector, v), will cause a radial (centripetal) acceleration of magnitude

$$a = v^2/r$$

Because the radius (r) of any turn or loop is large, the radial acceleration is physiologically equivalent to linear acceleration. Aircraft crew are so oriented that the acceleration vector resulting from lift is in the foot-to-head direction. This leads to a downward (head-to-foot) gravito-inertial G force that is termed $+G_z$, according to the following convention:

G force direction	Terminology
Backward (front-to-back)	$+G_x$
Forward (back-to-front)	$-G_x$
Transverse < to the left	$+G_y$
Transverse > to the right	$-G_y$
Downward (head-to-foot)	$+G_z$
Upward (foot-to-head)	$-G_z$

These forces can be colorfully described as; "eyeballs in" for $+G_x$, "eyeballs left" for $+G_y$, "eyeballs down" for $+G_z$, and so on.

III. Increased Foot-to-Head Acceleration ($+G_z$)

In 1919, a pilot reported alarming incapacitation while attempting to discover the smallest turning circle of a Sopwith triplane. He noted a "graying of the sky," fainted, and woke up flying over a village a mile away from the site of his experiment (Clark et al., 1961). He was exposed to $+4.5G_z$. The incapacitation he described is still a major cause of flying incidents and fatal accidents (Burton and Whinnery, 1985).

Large centrifuges were developed in the 1930s to explore human acceleration tolerance. A centrifuge rotating at 22 rpm will provide 5G radial acceleration in a gondola 30 ft from the axis. This radial component, and the normal 1G gravitational component result in $+5.1G$ at that point.

A. Normal Human Tolerance of $+G_z$

Impairment of vision ranging from "grayout" (peripheral vision loss) to total "blackout" limit the aviator's tolerance of $+G_z$, and are generally used as the physiological endpoints in acceleration research. Useful plots of G tolerance as a function of time were developed using these endpoints (Fig. 1). There is, however, marked intra- and intersubject variation in $+G_z$ tolerance. Neither centrifuge data nor the onset of visual symptoms can provide safe operational limits. Unconsciousness can occur at below $+4G_z$, and it is likely to occur without warning at high $+G_z$ onset rates (Burton and Whinnery, 1985). Although circulatory disturbances obviously limit $+G_z$ tolerance, we will see that they should be considered in the context of other thoracic effects.

B. Lung Function During Increased $+G_z$

The sensitivity of the pulmonary circulation to $+G_z$ has been recognized for many years (Gauer and Bondurant, 1961), but until recently, there has been less awareness of the marked effects of $+G_z$ on regional lung volumes, ventilation, and pleural pressure. Interest in the effects of $+G_z$ on the lung was increased when respiratory symptoms were described in pilots who breathed oxygen while exposed to $+G_z$ (Langdon and Reynolds, 1961).

Distribution of Ventilation

Morphometric studies in dogs showed that the gradient in alveolar size that is present at $+1G_z$ is further increased on exposure to $+G_z$ (Glazier and Hughes, 1968). The gradient is highlighted if an abdominal binder is used to prevent excessive descent of the diaphragm. In humans, the normal

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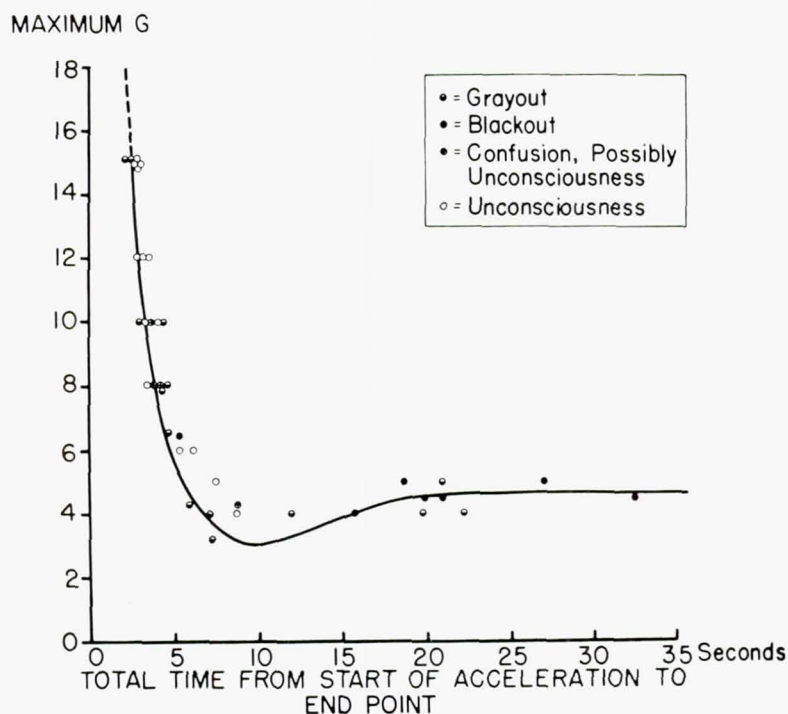


Figure 1 Normal human tolerance to $+G_z$. Data collected from a number of sources by Stoll (1956). Notice the correspondence between the dip in tolerance and the minimum systemic arterial pressure seen in Figure 2 (published with permission).

topographic gradient in ventilation seen at $+1G_z$ is accentuated as G_z increases. Airways closure also increases, especially when descent of the diaphragm is prevented by an inflated abdominal G-suit bladder and can occur during tidal breathing as the expiratory reserve volume is reduced (Glaister, 1970, 1977).

Distribution of Perfusion

Regional pulmonary blood flow in the lung is critically dependent on the local pulmonary arterial and venous pressures (P_a , P_v), which are subject to gravitational gradients, and on alveolar pressure (P_A), which is not (except possibly beyond closed airways). These gradients lead to three distinct zones in the lung at $+1G_z$ (West et al., 1964).

Zone 1 ($P_A > P_a > P_v$), in which perfusion is absent, is normally restricted to the lung apices.

Zone 2 ($P_a > P_A > P_v$), in which perfusion depends upon the arterial-alveolar pressure difference and, thus, changes steeply down the lung.

Zone 3 ($P_a > P_v > P_A$), in which perfusion depends on the arterial-venous pressure difference, which is large and constant.

The topographic distribution of these zones should depend upon the value and orientation of G , and should be readily predictable from the current P_a , P_v , and P_A values. Glaister (1970) measured P_a at $+1$ to $+2.8G_z$ in human subjects. He found that P_a was linearly related to $+G_z$, as it must be in a hydrostatic system in which the pressure is remaining constant at one point (the "hydrostatic indifference" point). The calculated hydrostatic indifference (constant pressure) point here was 5 cm below the pulmonary trunk. He assumed that transvascular pressure across the pulmonary veins in the hilum would not fall below zero and, thereby, estimated the topographic distribution of venous pressure. Zone 1 was estimated to occupy the upper half of the lung at $+3G_z$.

Permutt (1967) pointed out that the greatest changes in zone 1 should occur just above the $+G_z$ value where it first appears because the height (h) perfused above the point of hydrostatic indifference, at a constant pressure head (P) is inversely related to $+G_z$:

$$h = P/(\rho \cdot g \cdot G_z)$$

where ρ is the specific gravity of blood.

Zone 2 can be predicted to be short: $P_a > P_A > P_v$ is true from the point at which $P_A = P_a$ to the point where $P_A = P_v$. The difference in height (h) between these points is:

$$h = (P_a - P_v)/(\rho \cdot g \cdot G_z).$$

It can be seen that zone 2 height should be only 3.3 cm at $+3G_z$ (versus 10 cm at $+1G_z$) if $P_a - P_v$ is 7.6 torr. As a consequence, most of the lung should be either in the zone 1 or zone 3 state, during increased $+G_z$.

Bryan et al. (1965) performed ^{131}I -labeled macroaggregated albumin perfusion scans during $+1$ to $+4G_z$ accelerations. That study, along with later ^{133}Xe studies, confirmed the theoretical predictions in general, although zone 2 could not be clearly distinguished from zone 3, and there seemed to be a steady increase in perfusion down the lower lung regions. This may be so because compression of dependent alveoli would increase flow per unit lung volume (the measured quantity) at a constant flow per capillary unit (Glaister, 1977).

Distribution of Gas Exchange

There is a steady fall in the PaO_2 of dogs exposed to $+G_z$ while breathing air, especially if the otherwise marked descent of the diaphragm is prevented by abdominal binding (Glaister, 1968) or inflation of the abdominal bladder of a G-suit (Modell et al., 1985). Aviators strain, and abdominal bladders of G-suits inflate during $+G_z$, and both these actions limit diaphragm descent. Aviators become hypoxemic during $+G_z$ while breathing air (Barr, 1962). Although this hypoxemia could be simply consistent with the increased topographic inhomogeneity of both ventilation and perfusion, dependent airways closure is prominent, and the major cause of the hypoxemia is almost certainly the attendant population of extremely low ventilation/perfusion ratio (\dot{V}_A/\dot{Q}_c) units (Glaister, 1970).

The Oxygen Atelectasis Problem

The rate of absorption of gas from low \dot{V}_A/\dot{Q}_c units and the critical \dot{V}_A/\dot{Q}_c leading to atelectasis, both rise as the FIO_2 rises (Dantzker et al., 1975). Thus, the combination of a raised FIO_2 , a G-suit, and $+G_z$, predictably leads to basal atelectasis. Lower FIO_2 mixtures, and pressure breathing can be used to minimize this problem (Glaister, 1970; Shubrooks, 1973).

Pleural Pressure Gradients

Modell and Baumgardner (1984) found that apical (craniad) pleural pressures became increasingly negative with increasing $+G_z$ (craniocaudal **G** vector) in pigs and dogs. The basal (caudad) pleural pressures were near atmospheric during relaxation, but they became extremely high ($+20 \text{ cmH}_2\text{O}$ at $+5G_z$) with abdominal G-suit inflation. It seems that the lung can be virtually suspended by strongly negative apical pressures in the relaxed state, or it can be supported by massively positive diaphragmatic pleural (and abdominal) pressures with G-suit inflation. This implies that the pericardial pressure must also be markedly influenced by abdominal tone and G-suit status.

C. Cardiovascular Effects of $+G_z$

The principal hemodynamic effect of arising from the lying posture is a redistribution of venous volume, with approximately 500 ml of blood pooling in the legs (Blomqvist and Stone, 1985). However, the hemodynamic effects of increasing $+G_z$ are somewhat different, and direct arterial hydrostatic effects are of greater importance.

Direct Hydrostatic Effects

In 1946, Lambert and Wood showed that eye-level arterial pressure falls by about 32 torr/ G_z . This corresponds with a hydrostatic indifference point

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at heart level. They noted no visual disturbances with eye-level pressures above 50 torr. Systolic pressures of 20 torr (approximately the intraocular pressure) were associated with complete loss of vision.

Venous Return

Increasing $+G_z$ causes 12–50 ml/ G_z blood pooling in the legs. This initial blood pooling, which takes 25 sec, is followed by a slow increase in leg volume, with about 70 ml/ G_z accumulation in 5 min (Glaister, 1970). This is a modest volume when compared with the 500 ml that pools in the legs during the transition from lying to standing, and it probably reflects the difference between the filling of collapsed veins and the distention of them, with venoconstriction counteracting the latter process.

Many workers assume that splanchnic pooling occurs, but in humans transmural pressures in the abdomen do not increase. Rushmer (1947) found intra-abdominal pressure rose by 15 torr/ G_z , providing an approximate balance to the intravascular hydrostatic gradient. The situation may, however, be different in anesthetized animals with more relaxed abdominal muscles.

The blood redistribution resulting from increasing G_z might be expected to decrease the cardiac preload and the atrial and ventricular end-diastolic pressures. Before reviewing such data, it is important to stress that hydrostatic gradients cause major measurement errors at $+G_z$. Subjects, and the seats or harnesses used, sag under stress and, thus, the hydrostatic relationship of any catheter tip to a fixed transducer changes. This leads to a large apparent change in pressure (e.g., 3.9 torr/cm at $+5G_z$).

Glaister (1970), while studying a single subject, recorded a fall of right atrial pressure (referred to pulmonary trunk level) from zero to -7 torr at $+3G_z$. Peterson et al. (1977) measured left ventricular end-diastolic pressure (LVEDP) in lightly anesthetized, chronically instrumented dogs. At $+3G_z$, when aortic flow had fallen to 33% of the control value the LVEDP had fallen from $+1.4$ torr to -5 torr. Dye-dilution studies report an 18 to 24% decrease in cardiac output shortly after the onset of $+3G_z$ (Lindberg et al., 1960; Rosenhamer, 1967). Boutellier and coworkers (1985), obtained similar results using a CO_2 -rebreathing technique.

Reduction in cardiac output during exposure to $+G_z$ may be entirely due to simple loss of venous return and preload reduction. However, this explanation is as tentative as were similar early explanations of the effects of positive end-expiratory pressure (PEEP).

Possible Mechanical Heart-Lung Interactions

Peterson and associates (1977) found that although G-suit inflation could maintain LVEDP at higher than normal values during $+6G_z$ in their

permanently instrumented dogs, cardiac output remained well below the control value. This is a most interesting observation.

Total peripheral resistance was greatly elevated (217% of control—largely because of the occlusive effect of the G-suit), and this increase in afterload must have contributed to the low cardiac output. The authors did, however, speculate that high abdominal pressures generated by the G-suit may have increased pericardial pressure. When one considers the later measurements of basal pleural pressure made by Modell and Baumgardner (1984) with values of + 20 cmH₂O in G-suited dogs at +5G_z, these speculations seem increasingly relevant.

It now seems important to make direct transcardiac pressure measurements, or carefully follow chamber volumes, when studying cardiac performance at the limits of +G_z tolerance. Direct mechanical heart-lung interactions are likely, and it is possible that basal lung compression and airways closure may effectively embed the heart in a noncompliant tissue.

Reflex Effects

After an initial fall upon exposure to +G_z, head-level arterial pressure climbs back toward normal, and there is an associated tachycardia. After return to +1G_z, there is a brief arterial pressure overshoot. Similar changes are seen in the dog femoral arterial tracing in Figure 2 (Glaister, 1970), and the response can be seen to differ from that seen in the passive pulmonary circulatory system.

Carotid baroreceptor activity is probably largely responsible for the secondary rise (Peterson and Bishop, 1979; Abboud et al., 1979). Both cardioacceleration and active arteriolar vasoconstriction occur. Although splanchnic vasoconstriction is related to falling carotid pressure, probably the decreased activity of the low-pressure central mechanoreceptors causes vasoconstriction in limb muscle beds (Donald and Shepherd, 1978; Blomqvist and Stone, 1985).

Venous return is also augmented. Salzman and Leverett (1956) demonstrated dog saphenous vein constriction during +G_z, and Hiatt et al. (1958) showed similar constriction in human forearm and saphenous veins. Venomotor activity is potentiated by high distending pressures (Öberg, 1967; Blomqvist and Stone, 1985). Whereas splanchnic veins probably constrict in response to arterial baroreceptor stimulation, cutaneous veins have been shown to constrict in response to active respiratory effort (Browse and Hardwick, 1969), such as occurs in both humans and pigs, in response to +G_z (discussed later).

D. Protective Measures

Straining Maneuvers

Early investigators soon learned that +G_z tolerance could be voluntarily extended. Lambert and Wood (1946) wrote:

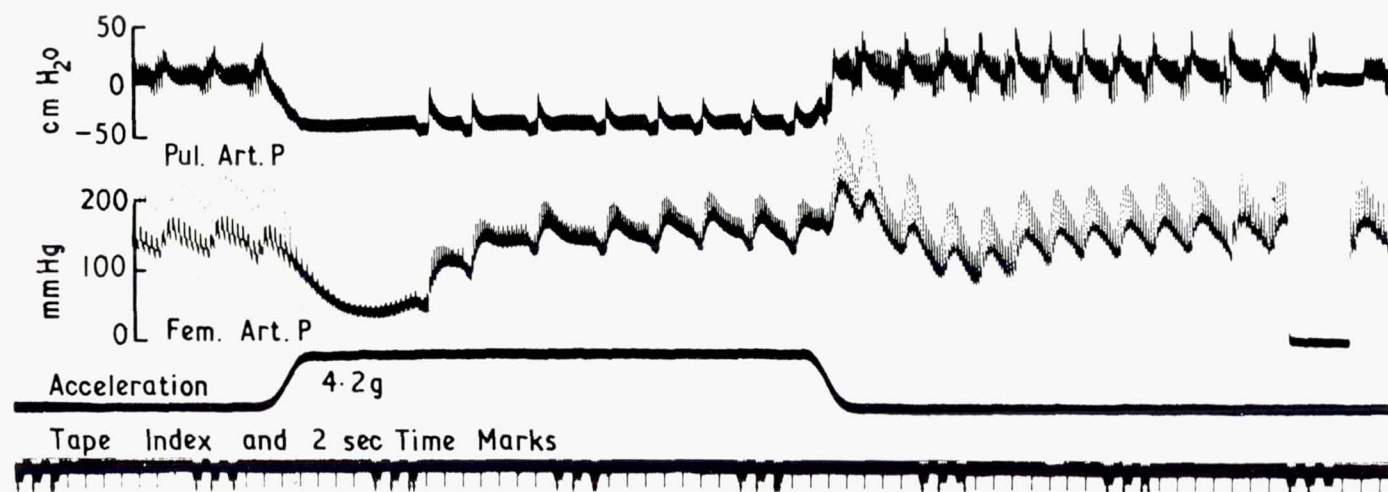


Figure 2 Records of pulmonary arterial pressure referenced to the level of the pulmonary trunk, and femoral arterial pressure referenced to the level of the left ventricle from an anesthetized dog exposed to +4.2G_z for 1 min (from Glaister, 1968, with permission).

Some of the straining procedures used by veteran pilots have been selected and refined on the basis of centrifuge studies to produce a very effective maneuver. This consists essentially of a series of rapidly repeated forced expirations against a partially closed glottis coordinated with muscular straining.

This maneuver was called the "M-1 anti-G straining maneuver." It now also involves crouching down in the seat as much as possible, and the glottis can be completely closed (Shubrooks and Leverett, 1973). Cohen (1983) found that the M-1 maneuver improved tolerance limits from a mean of $+3.2G_z$ in the relaxed state, to $+4.9G_z$. It is perhaps puzzling that performing a Valsalva-like maneuver, which has been used to decrease tolerance of $+1G_z$ (Howard et al., 1951), can improve $+G_z$ tolerance. The M-1 maneuver, however, includes some actions that are not generally performed when the Valsalva maneuver is performed in other settings.

The tensing of muscles is an important part of the M-1 maneuver and has major cardiovascular effects. Lohrbauer et al. (1972) demonstrated that protection rivaling that of the full M-1 maneuver (peripheral light loss at $+4.5G_z$ vs. $+3.6G_z$ relaxed) was afforded by sustaining a handgrip at 30–50% maximum while maintaining baseline intrathoracic and intragastric pressures. The handgrip produced a 25-torr mean arterial pressure elevation, associated with significant tachycardia, during control runs at $+1G_z$. In lightly anesthetized cats, muscle contraction increases blood pressure, heart rate, and contractility by reflexes mediated by group III and IV muscle afferents (Kaufman et al., 1984). Exercise evokes venoconstriction in humans probably by similar reflexes (Fortney et al., 1983).

It is instructive to compare the thoracic part of the M-1 maneuver with the normal Valsalva maneuver (Fig. 3). Eckberg (1980) has reviewed the latter maneuver in more detail.

Phase I

The initial rise in blood pressure has two potential sources. First, direct transmission of increased intrathoracic pressure to the arterial system; and second, an increased stroke volume resulting from the ventricular unloading effects of increased intrathoracic pressure. Note that phase I is associated with a slowing of the heart rate, as a result of carotid baroreceptor stimulation in the normal $+1G_z$ environment, and that this must reduce the rise in blood pressure.

At $+G$ this parasympathetic effect will be much less because carotid level blood pressure is much lowered by a direct hydrostatic effect. Burns et al. (1986) studied G-suit-equipped conscious miniature swine, exposed to $+G_z$. Grunting momentarily raised esophageal pressure up to 60 torr

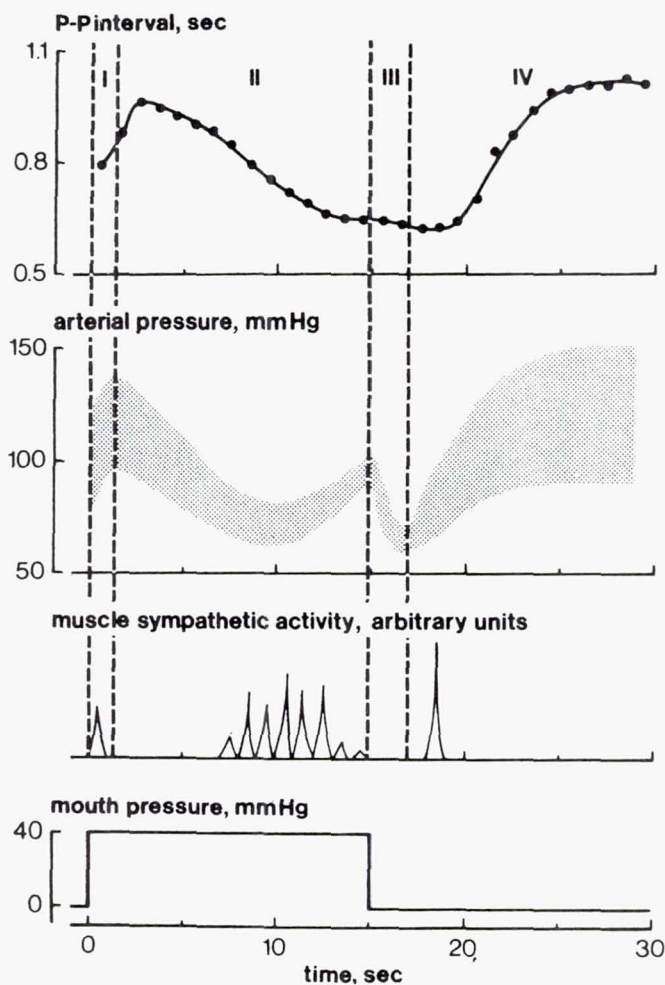


Figure 3 Schematic representation of a normal response to the Valsalva maneuver. The four phases detailed in the text are indicated by Roman numerals (from Eckberg, 1980, with permission).

and eye-level arterial pressure almost the same amount (Fig. 4). Thus, aortic transmural pressure remained approximately constant.

Phase II

The secondary fall in blood pressure is due to a decreased venous return and, thus, shortly after, a decrease in cardiac output.

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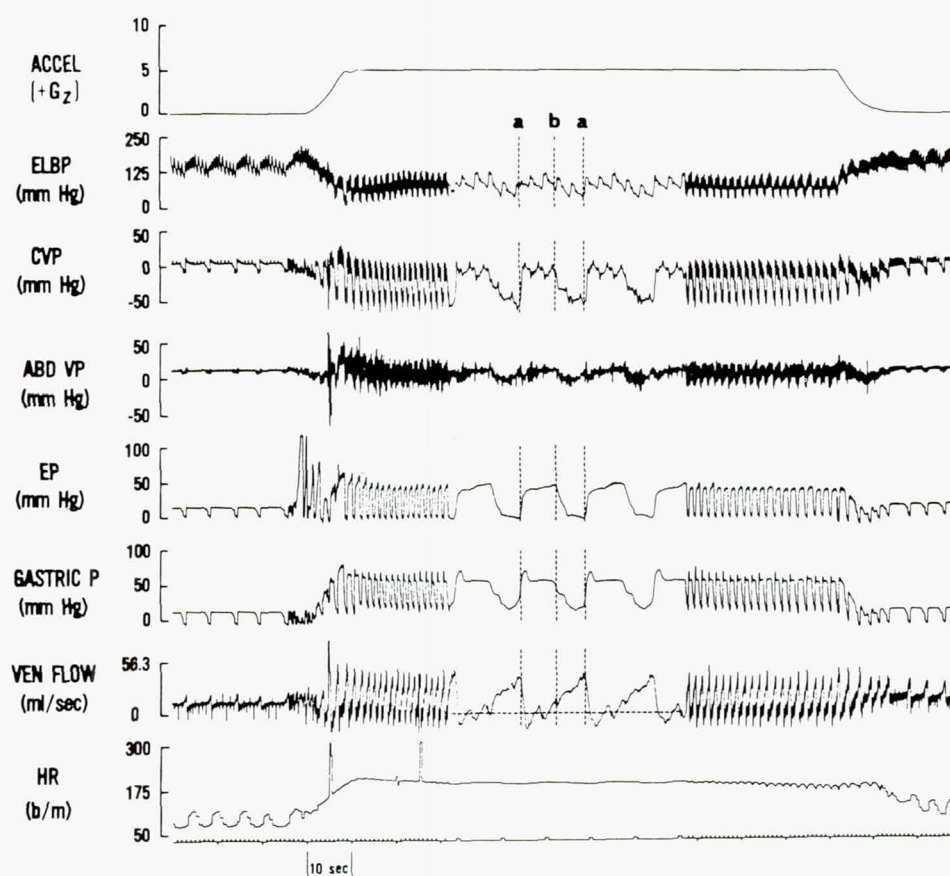


Figure 4 Influence of repetitive anti-G straining as evidenced by esophageal pressure (EP) changes in a G-suited miniature swine exposed to +G_z as indicated. The other measurements are: ELBP, eye-level blood pressure; CVP, central venous pressure recorded in the superior vena cava; ABD VP, abdominal venous pressure recorded in the abdominal vena cava; GASTRIC P, gastric pressure recorded in the stomach; VEN FLOW, venous blood flow in the inferior vena cava recorded at the diaphragm; HR, heart rate. Note the rise in ELBP (a to b) in phase with the EP rise also corresponding with a fall in venous flow. Note also the sharp increase in venous flow across the diaphragm during the relaxation phase of the maneuver (from Burns et al., 1986, with permission).

Rushmer (1947) noted that blood pressures fell more dramatically when Valsalva maneuvers were performed at high lung volumes than at the lower volume of the M-1 maneuver. It now seems that the heart-lung interactions that are seen at near-total lung capacity (such as increased right ventricular afterload at high transpulmonary pressure) may account for some of this difference.

The blood pressure fall is arrested by the onset of tachycardia and increased sympathetic arteriolar constriction. At high $+G_z$ there is preexisting tachycardia and sympathetic outflow before the M-1 maneuver is started, and muscular straining also causes sympathetic activation. Phase II falls are modest during M-1 maneuvers (Shubrooks and Leverett, 1973). However, the important feature of the M-1 maneuver is that it is "brief and repetitive" (Lambert and Wood, 1946) and, thus, the (modest) falls seen in longer strains (Fig. 5) are avoided (see Fig. 4).

Phase III

The sudden drop in arterial pressure as mouth and, hence, pleural pressure drops is due to both a direct hydrostatic effect and a sudden increase in left ventricular afterload.

Inferior vena cava flow (see Fig. 4) can be seen to be increased during phase III, presumably because the abdominal venous pressure has risen during phases I and II as a result of "damming," abdominal muscle tone and venoconstriction (Burns et al., 1986).

Phase IV

Phase IV is the overshoot phase. This is thought to be due to restoration of cardiac output into a constricted vascular bed.

In the M-1 maneuver performed at $+G_z$, the bradycardia should not be pronounced, because the carotid level pressure rise is only toward normal. Repetitive M-1 maneuvers obscure this phase, but they are, no doubt, potentiated in their pressor effect by the underlying vasoconstriction.

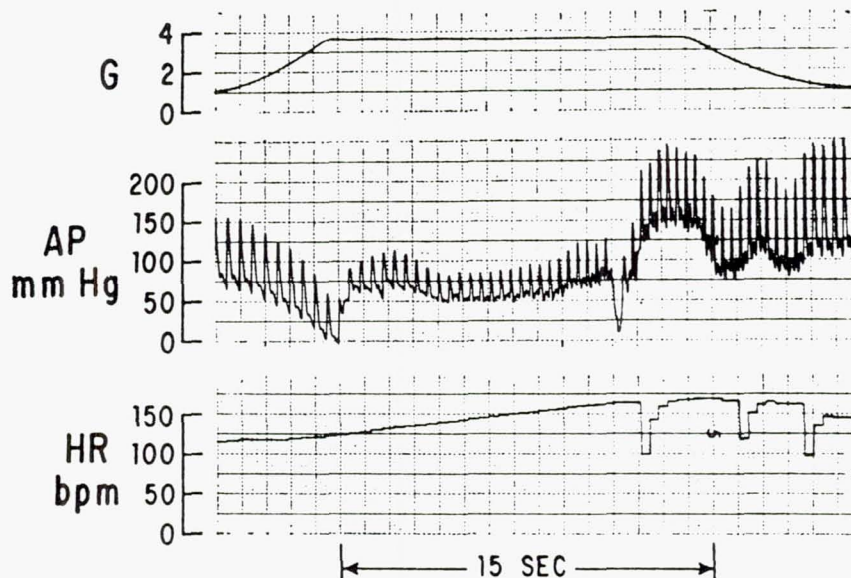
In summary, it is known that the M-1 and similar straining maneuvers consistently elevate blood pressure during $+G_z$. Straining enhances vasoconstriction and elevates the mean systemic pressure. The usual parasympathetic depressor reflexes are not prominent because the carotid level arterial pressure is low. The adverse effects of raised intrathoracic pressure (a fall in cardiac output and arterial pressure) can be minimized by using rapid repetitive M-1 maneuvers, where venous return and arterial pressure elevation occur in opposite phases (see Fig. 4).

G-Suit Effects

Early physiologists recognized that equal and opposite hydrostatic counter-pressure would prevent the redistribution of blood during $+G_z$. Franks in

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B. VALSALVA 3.7 G

Figure 5 Arterial pressure referenced to eye-level and heart rate response in humans to the Valsalva maneuver at $+3.7G_z$ without the use of a G-suit. Note the small secondary fall in blood pressure after the initial rise. The end of the maneuver is clearly indicated by the phase III fall in arterial pressure (from Shubrooks and Leverett, 1973, with permission).

1940, developed a water-filled suit of inelastic material, which extended tolerance by approximately $+1.7G_z$ (Leverett et al., 1961). Later, Webb and Gray (1960) established that total immersion made $+9G_z$ comfortable (and up to $+31G_z$ tolerable) provided breathing pressure was appropriate.

It was soon apparent that pneumatic counterpressure was more practical than immersion and that it was not necessary to simulate the hydrostatic gradient. The standard U.S. Air Force anti-G suit (USAF CSU-12/P) is representative of the current technology, which dates back to the late 1940s (Wood, 1987). Five interconnecting bladders, over the calves, thighs, and abdomen, are incorporated in a snugly fitted, adjustable, cut-away garment that is worn over the flying suit. An automatic valve admits air through the abdominal bladder. As $+2G_z$ is well tolerated, and the filling and emptying of the suit during normal turbulence and maneuvering is rather distracting, the valve is generally set to operate only at more than

+2G_z. A typical protocol is linear pressurization at 76 torr/G_z (1.5 psi/G_z). Whereas +G_z protection on the centrifuge bears a linear relationship to applied suit pressure, extreme discomfort precludes the practical use more than 414 torr (8 psi) (Hrebien and Hendler, 1985).

The mechanism of blood pressure elevation by G-suits is controversial. The contenders are an increase in systemic venous return and an increase in total systemic resistance (TSR). The controversy may well be due to the predominance of one or the other mechanism under different conditions.

Gaffney et al. (1981) pointed out that hypovolemic subjects who benefit from medical antishock trousers (MAST) do not have a large splanchnic and lower-limb venous volume for central translocation. They found that MAST increased TSR 48% and cardiac output only 18% in their supine normal subjects.

Seaworth et al. (1985) used echocardiography to establish that G-suit inflation in the upright posture significantly increases end-diastolic volume and cardiac output and actually produces a fall in TSR.

Inflation of G-suits at +1G_z does not necessarily provide good evidence of mechanisms at +G_z. Animal studies of inflation during +G_z stress occur in the setting of established vasoconstriction. Lightly anesthetized dogs were centrifuged by Peterson and coworkers (1977) and developed very high TSRs (124% above control) at +3G_z. G-suit inflation to 76 torr partially restored the cardiac output, relieved the hypotension, and caused a net fall in TSR (to 60% above control). Thus, G-suit inflation must have improved the venous return.

It is dangerous to apply animal data to humans uncritically. Pigs and dogs have relatively much less blood volume in their hind limbs, and abdominal counterpressure is likely to play a greater part in the G-suit effect, especially if anesthesia is used. Conscious, permanently instrumented miniature swine are presently the best animal model, especially because they grunt and strain during +G_z in a very human fashion.

Miniature swine were used in the recent study of Burns et al. (1986). Hemodynamic data from that study suggests that both an increase in TSR and improved venous return are important G-suit mechanisms. The study also documented two other important effects. The G-suit increases the efficacy of the M-1-like straining by providing a "platform" against which respiratory muscles can strain. Elevation of abdominal pressure also prevents descent of the heart. Glaister (1970) has previously described this effect in humans in whom a 2-cm descent at +4G_z (causing a 6-torr eye-level blood pressure fall) can be prevented by G-suit inflation.

Pressure Breathing

It will be shown in the next section that pressure breathing elevates arterial blood pressure. Pressure breathing is associated with expiratory straining and is as effective as the M-1 maneuver in providing $+G_z$ protection, particularly when used with a G-suit. The mechanics involved are similar to those seen in the M-1 maneuver and are discussed by Shubrooks (1973).

Altering the G Axis

Acceleration is tolerated much better lying down (G_x). Aircraft seats have been developed that elevate the pelvis and legs during $+G_z$ acceleration, but this does little to address the critical problem: the hydrostatic pressure difference between head and heart.

Eventually, the wisdom of adopting the prone or supine position in fighter aircraft may become inescapable, and the interesting problem of high sustained $+G_z$ will become rather academic. This is now anything but true with rapidly escalating aircraft performance, an unchanging system of $+G_z$ orientation (dating back to the 1900s), and with protective systems largely unchanged since the 1940s (Wood, 1987).

IV. Pressure Breathing

A. Altitude Tolerance

The highest safe altitude when breathing pure oxygen at ambient pressure is about 40,000 ft (barometric pressure 141 torr, PAO_2 54 torr with a $PACO_2$ of 40 torr).

This limit was extended during World War II by using pressure breathing (PB) to maintain a higher-than-atmospheric pressure in the lungs (Barach et al., 1947). An airway pressure of 141 torr at 45,000 ft requires mask pressure of 30 torr, which is the practical limit of sustained human tolerance (Ernsting, 1966). A record 1961 glider flight to 46,266 ft still stands for that reason.

Pressure breathing, as a means of extending altitude tolerance, is now generally used only in depressurization emergencies. Modern aircraft can descend to below 40,000 ft extremely rapidly, and PB is required for only 2 or 3 minutes, at the most. Higher airway pressures (or some hypoxia) can be tolerated for such short periods, and simple mask PB systems suffice to 50,000 ft (Table 1).

United States military aviators use pressure suits when there is a risk of exposure to higher than 50,000 ft. The British, however, extend tolerance to PB using a stepwise application of counterpressure, tailored to the aircraft role. Their approach is summarized in Table 1 and is noteworthy

Table 1 Altitude Limits during Pressure Breathing

System	Mask, Helmet, Mask, jerkin, jerkin, G-suit		Mask, Helmet, jerkin, G-suit, arm extensions	
	Mask	jerkin	G-suit	G-suit
Mouth pressure (torr)	30	50	60	107
Maximum altitude (1000 ft)	45	50	56	70
Maximum altitude (torr)	111	87	65	34

Source: after Ernsting, 1966

for its use of trunk counterpressure. Ernsting (1966) documents the extensive investigation that led to this system.

The Royal Air Force (RAF) pressure jerkin is a development of a wartime Canadian pressure vest in which mask pressure was applied to bladders in a tight-fitting waistcoat. Pressure vests of this type were extensively investigated in the United States in the 1940s, and it was recognized that an abdominal extension was required to prevent excessive descent of the diaphragm at high breathing pressures. Drury et al. (1947) found that this combined counterpressure allowed airway pressures of 45 torr to be tolerated for 10 to 20 min. The addition of a G-suit allowed subjects to tolerate 60 torr for as long as 30 min (Ernsting, 1966).

At high levels of PB, a pressure helmet is required to prevent extreme distension of the upper respiratory tract and severe pain that can cause sudden collapse. Arm pain from venous engorgement can be similarly incapacitating above 70,000 ft; therefore, arm counterpressure is required (see Table 1). Once this point is reached, the system is roughly equivalent to a "partial-pressure suit" with counterpressure over the entire body, at which point the cardiorespiratory stress of PB has largely disappeared.

B. Effects of Pressure Breathing on Lung Volume

An airway pressure of 20 torr (approx. 27 cmH₂O) will almost fully inflate the lungs of a relaxed subject. However, subjects maintain expiratory effort in response to the increased pressure. Despite this, tidal volume increases slightly, and expiratory reserve volume (ERV) increases markedly with increasing positive mask pressure, until at 30 torr (41 cmH₂O) the ERV has increased to over 80% of total lung capacity (TLC), and the inspiratory reserve volume is virtually zero. Pressure breathing at 30 torr can be tolerated for about 10 to 20 min, beyond which exhalation becomes exhausting and collapse can occur (Ernsting, 1966).

Trunk counterpressure is dramatically effective in preventing lung overdistension and easing the work of breathing. The RAF jerkin allows

only minimal increases in lung volume, which are largely attributable to a reduction in thoracic blood volume (Ernsting, 1966).

C. Cardiovascular Effects of Pressure Breathing

Findings from clinical and animal investigations of the effects of positive end-expiratory pressure (PEEP) should be applied with considerable caution in the aviation setting. Aviators resist lung overinflation and have learned to tense muscles strongly under the stresses of PB and $+G_z$. Direct investigations, however historic, should be studied first.

Peripheral Venous Engorgement

The thrust of many early investigations was to document the displacement of blood to the periphery. It was found that it took about 20 sec from the onset of PB for the peripheral venous pressure to plateau at a level approximately equivalent to the pleural pressure. Limb volume increased as venous pressure increased at about $0.018 \text{ ml} \cdot 100 \text{ ml} \cdot \text{torr}$ (Ernsting, 1966). Ernsting calculated that the immediate shift away from the trunk was 190 ml at a breathing pressure of 30 torr, which was consistent with the 300 ml shift that Henry (1951) had estimated at 40 torr. Ernsting's plethysmographic studies showed that there was a continuing slow increase in limb volume, doubling the fluid shift in 5 min.

This redistribution of blood is small in comparison with that seen in supine studies. Fenn et al. (1947) showed that PB at 20 torr displaced 500 ml of blood in supine subjects on a teeter board. This difference is analogous to the much larger shift of blood that occurs on going from the supine to erect position, than on going from $+1G_z$ to $+2G_z$. It probably represents the difference between filling an unstressed venous reservoir and distending an elastic one. Furthermore, pressure breathing causes active venoconstriction. Ernsting found that plethysmographic increases in limb volume were consistently less than those occasioned by equal local rises in venous pressure and that this difference was abolished by nerve blocks. Thus, there is a similarity to the Valsalva maneuver. The high tolerance of PB by aviators probably is due to venoconstriction and abdominal straining, as well as to vigorous use of the expiratory muscles.

Right Transatrial Pressure

Direct measurements of right transatrial pressures during aviators PB without chest counterpressure have not been made. However, an interesting pattern emerges when data from other sources is considered. Scharf et al. (1977) studied right transatrial pressure in dogs during PEEP, comparing a group with high lung volumes with a group with controlled

lung volumes (by use of pneumothoraces). The first group showed a rise, the second a fall in right transatrial pressure.

Cassidy et al. (1979), observed substantial increases in right transatrial pressure in normal subjects during relatively modest PEEP (7.5 torr). The subjects had been trained to relax, allowing their lung volumes to increase. Ernsting (1966) observed a fall in right transatrial pressure during PB in subjects wearing pressure jerkins. Earlier, Cournand et al. (1948) had observed a fall in right transatrial pressure during simple pressure breathing. They had, however, selected *patients with pneumothoraces* to enable accurate pleural pressure measurements. In retrospect, one can see that their anomalous results fit nicely into the pattern: PB causes a rise in right transatrial pressure if lung volume increases sufficiently.

It is not clear that aviators' PB at 7.5 torr would produce an increase in right transatrial pressure similar to that seen by Cassidy et al. (1979). At that pressure, aviators largely resist increases in lung volume (Ernsting, 1966). It seems likely that at the higher pressures used in aviation, right transatrial pressure would rise, especially toward the limit of tolerance, as lung volumes increase. Heart-lung interactions could be prominent under these conditions. Lung volumes may be sufficient to affect apparent ventricular performance (Culver et al., 1981), right ventricular afterload (Whittenberger et al., 1960), or trigger reflex cardiovascular depression (Cassidy, 1984; Ashton and Cassidy, 1985).

Effect on Arterial Blood Pressure

Aviation workers are especially interested in the acute transient blood pressure responses to the sudden onset of PB, as during both acceleration stress and rapid decompression, these transient responses might lead to sudden incapacitation. Ernsting (1966) reviewed these transient changes, which are similar to those seen with the Valsalva maneuver. There is an initial increase in arterial pressure paralleling the increase in pleural pressure, which is maintained for several seconds, followed by a fall in both mean and pulse pressure, and then a second rise, with the pressure stabilizing at a new raised value. Ernsting related this second rise to a partial restoration of venous return after filling of the venous capacitance. The prominence of rising mean systemic pressure must depend on venoconstriction, straining, and the presence or absence of a G-suit.

Ernsting (1966) explored the relationship between PB pressure and blood pressure in subjects wearing helmets. Pressure breathing caused the arterial pressure to rise by approximately 50% of the airway pressure with no counterpressure, 70% with chest counterpressure, 90% with trunk counterpressure, and 110% with trunk and lower-limb counterpressure. These results must be interpreted with caution when applied to mask PB.

Ernsting noted that the helmet bladders applied airway pressure over the carotid arteries and that deflation of the appropriate bladders caused the arterial pressure to fall by about 50% of the previously applied counter-pressure. This suggests that mask PB should have less effect on arterial pressure.

Cassidy et al. (1979) reported average systolic and diastolic increases of approximately 5 torr during 1 hr of 7.5-torr PEEP. Because PB can increase arterial blood pressure, it is not surprising that it is now being investigated as a means of protection against $+G_z$. Shubrooks (1973) showed that 20 to 40 torr mask PB increased arterial pressure by approximately 25 torr at approximately $+3.5G_z$. As any carotid reflex that might limit the increase in blood pressure at $+1G_z$ would be minimized at $+G_z$ by the hydrostatic effect, one would expect mask PB pressor responses at $+G_z$ to be similar to the helmet PB response seen by Ernsting (1966), on the ground.

Effect on Left Ventricular Afterload

Left ventricular afterload is determined by the left ventricular transmural pressure (Permutt, 1973). This pressure is reduced in anesthetized dogs receiving PEEP (Scharf et al., 1980) where the rise in blood pressure is minimal and should be reduced as long as the rise in blood pressure is less than the rise in pleural pressure. Ernsting's data suggests that this holds true in aviators' PB unless total counterpressure is applied.

Pressure-Breathing Syncope

Ernsting (1966) noted that syncope usually supervened at the limit of tolerance of pressure breathing (Fig. 6). He described a biphasic fall in blood pressure. First there was a slow fall associated with an increasing tachycardia, facial pallor, and sweating. Then there was an abrupt fall in blood pressure, with bradycardia, and syncope. Ernsting noted a marked decrease in peripheral resistance of the forearm at that time. He suggested that the very small left ventricular end-systolic volume would stimulate intracardiac receptors, thus, causing reflex peripheral vasodilation and bradycardia (Sharpey-Schafer et al., 1958).

The major effect of pressure breathing on left ventricular afterload must lead to very low ventricular volumes in the face of falling arterial pressure. The mechanism described by Ernsting (1966) very closely resembles the mechanism suggested by Epstein et al. (1968) for vasovagal syncope, that was based on observations during head-up tilt and lower-body negative pressure (LBNP).

Ernsting's observations were made on subjects in pressure jerkins. In this situation, the syncope is obviously analogous to "vasovagal" fainting

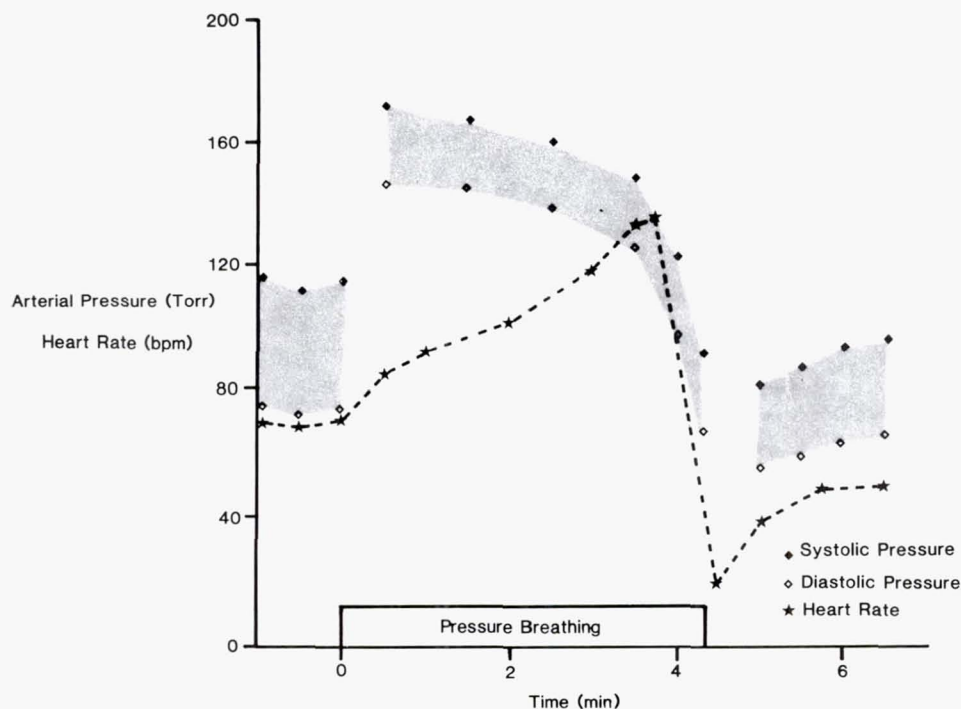


Figure 6 Pressure-breathing syncope: heart rate and blood pressure response to 80 torr of pressure breathing with chest counterpressure. Note the biphasic fall in blood pressure and the reversal of tachycardia in the second phase (from Ernsting, 1966; the original was first published in AGARD AG 106, dated 1966, by the Advisory Group for Aerospace Research and Development, North Atlantic Treaty Organization, AGARD/NATO).

seen in other settings. The syncope seen with simple mask PB may also be analogous, but other heart-lung interactions might play a larger role because respiratory muscle exhaustion could lead to extreme lung hyperinflation.

Effect on Cardiac Output

It is instructive to compare the reported effects of PB on cardiac output in different experimental settings (Table 2).

It can be seen that cardiac outputs are still 50% of normal in anesthetized dogs at 15 torr (20 cmH₂O) PB. This is possible only if the normal venous return curve shifts to the right, presumably because of

Table 2 Effect of Pressure Breathing on Cardiac Output

Experimental conditions	Change in cardiac output
Closed-chest dogs (Scharf et al., 1977)	50% fall at 15-torr PB
Relaxed humans (Cassidy et al., 1979)	20% fall at 7.5-torr PB
Aviators (Ernsting, 1965)	40% fall at 30-torr PB
Aviators, trunk counterpressure (Ernsting, 1965)	30% fall at 30-torr PB

sympathetic venoconstriction, and because descent of the diaphragm raises the abdominal pressure, shifting blood away from the splanchnic reservoir (Scharf et al., 1977). The relaxed human response to PB can be seen to be similar. Aviators' PB (nonrelaxed), can be seen to cause much less reduction in cardiac output. Straining is known to enhance venoconstriction (Browse and Hardwick, 1969), and aviators strain and maintain higher abdominal than pleural pressures to avoid lung expansion. Increased abdominal pressure increases cardiac output during PEEP (Scharf and Ingram, 1977), probably because of mobilization of blood in the splanchnic reservoir. The right shift of the venous return curve under these conditions must be large. Trunk counterpressure can be seen to lessen the decrease in cardiac output, as is consistent with current understanding of the influence of lung inflation on the right-side heart hemodynamics and with the added effect that abdominal counterpressure must have on mean systemic pressure.

D. Neurohormonal Effects

Modern aviation application of PB is of very short duration, but PB was used for prolonged periods in the 1940s and urine output fell (Drury et al., 1947). Further investigation showed that negative-pressure breathing had the opposite effect (Gauer et al., 1954; Sieker et al., 1954). This work led directly to the recognition that volume receptors must exist in the atria and pulmonary venous system (Gauer and Henry, 1963; Meehan, 1986). This topic is of great interest to investigators of the effects of microgravity and will be discussed in the next section.

V. Spaceflight (Microgravity)

Dietlein (1977) summarized the findings of the Skylab investigation of the human response to prolonged weightlessness. There was:

1. A marked shift of fluid from the lower to the upper half of the body
2. An increasing sensitivity to lower-body negative pressure during the flight
3. A small loss of circulating blood volume
4. A maintained exercise capacity in space
5. Postflight orthostatic intolerance, decreased exercise capacity while upright, and decreased cardiac size with normal contractility

It was concluded that circulatory and renal responses to an increase in central blood volume, caused by the headward fluid shift in microgravity (O-G), must play a large part in the "cardiovascular deconditioning" seen on return to $+G_z$. Parallels have been drawn to the changes seen during exposure to bed rest, supine head-down tilt, and immersion in water.

Unfortunately, many of the early hemodynamic and renal responses that are seen in these ground-based models have not been observed in microgravity. This may be because they are short-lived. Most of the cardiovascular effects seen at the onset of head-down tilt were undetectable after 6 hr (Nixon et al., 1979). Measurements were scanty in the first few days during the Skylab program, and very little relevant data has been collected since. There are other difficulties. Crews are supine and, thus, adapting to a lessened gravitational load for at least 2 hr before launch. The nausea, anorexia, and vomiting of "space sickness" can have devastating effects on any study of circulatory, neuroendocrine, or renal function during the first 2 to 3 days of flight.

In this section, we focus on the acute changes seen during the first week of weightlessness, and immediately after return to $+1G_z$. Current understanding still depends on ground-based simulations (immersion, bed rest, and head-down tilt). Blomqvist and Stone (1985), Sandler (1980), Levy and Talbot (1983a), and Greenleaf (1984) have reviewed these topics more extensively.

In this volume concerning heart-lung interactions, it is noteworthy that many of the proposed acute circulatory effects of weightlessness involve small increases in *transmural* pressures in the thoracic cardiovascular structures. It is appropriate to consider the effects of weightlessness on the lung before discussing cardiovascular changes because if weightlessness, or its simulations, causes changes in lung volumes and pleural pressure topography (as seems likely), then measured vascular pressures should be

related to the new measured, or at least calculated, pleural pressures. It will become apparent that few of the studies that follow have adequately addressed this problem: small changes in CVP, for instance, are extremely difficult to interpret without knowledge of the concomitant changes in resting lung volumes and pleural pressure, as the subject goes from $+G_z$ to O-G, or becomes immersed, recumbent, or is tilted head down.

A. Acute Effects of Microgravity

Regional Effects on Lung Function

The large topographic gradients in alveolar size, ventilation, and pulmonary blood flow that are seen at $+1G_z$ (Glazier et al., 1967; West et al., 1964) should virtually disappear in microgravity (Glaister, 1977). Virtual weightlessness can be sustained for 20 to 40 sec in aircraft, enabling studies of the effect of short-term microgravity on lung function.

Ventilation

Michels and West (1978) performed combined single-breath nitrogen-argon bolus washout tests during LearJet parabolas. The cardiogenic oscillations of both resident and bolus inert gases virtually disappeared; thus, topographic gradients in alveolar size and ventilation had decreased markedly (Fig. 7).

There were small terminal nitrogen rises (phase IV) on many of the LearJet O-G tracings suggesting the presence of minor nongravitational inhomogeneity of airways closure, as predicted by Glaister (1977). It is possible that vascular engorgement is, at least partly, responsible for this effect. Head-out immersion in water causes a cephalad blood redistribution and an increase in closing volume. It has been shown that much of this increase can be prevented by cuffing the limbs, thereby decreasing central vascular engorgement (Bondi et al., 1976).

The suggestion has been made that the diaphragm should adopt a more cephalad position at O-G and, thus, FRC may be reduced. An increase in thoracic blood volume may possibly account for the 10% reduction in vital capacity that was seen in Skylab-4 crews in orbit (Levy and Talbot, 1983b).

It seems highly unlikely that gas exchange will be compromised because of any of these effects. Rather, it seems that ventilation should become strikingly more uniform in the microgravity environment. Furthermore, because vascular engorgement should decrease with adaptation, any terminal airways closure and vital capacity reduction may also decrease.

Perfusion

Stone et al. (1965) injected ^{131}I -labeled, macroaggregated albumin intravenously during parabolic flight and scanned immediately postflight.

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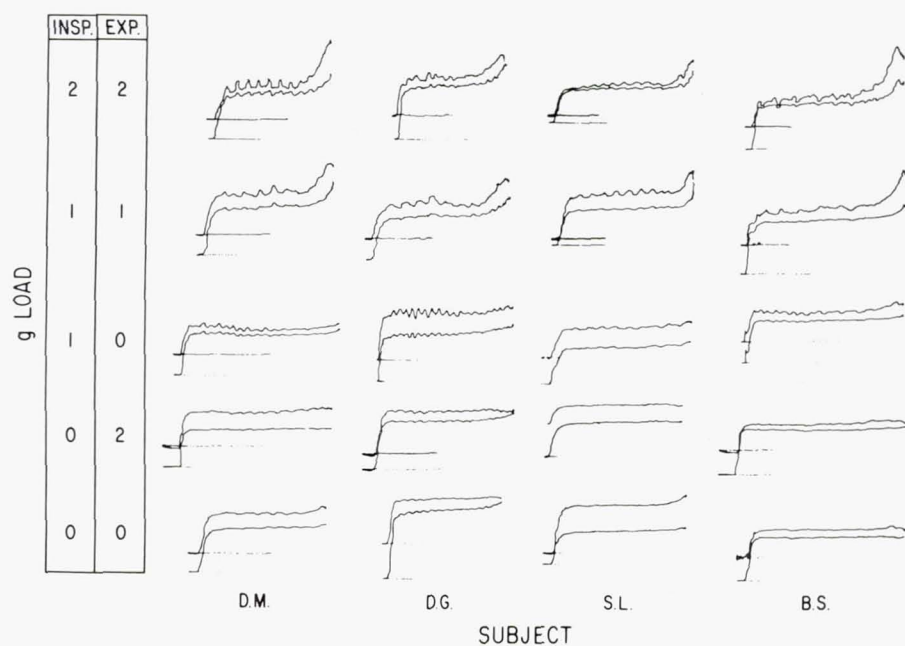


Figure 7 Comparison of single-breath washouts from four subjects (columns) at various gravitational loads (rows) with inspiratory and expiratory G_z levels indicated on the left. For each, the upper curves are Ar-bolus washouts, and the lower are N_2 washouts. Expired volume is plotted along the abscissa and normalized gas concentrations are plotted along the ordinate.

Notice the virtual absence of cardiogenic oscillations in phase III when inspiration is carried out at O-G and the strong dependence of the magnitude of the cardiogenic oscillations on inspired G_z level. Note the near disappearance of phase IV in some subjects at O-G (from Michels and West, 1978, with permission).

They found a considerable shift in blood flow toward the lung apices. Michels and West (1978) showed that cardiogenic oscillations of oxygen and carbon dioxide virtually disappeared at O-G, suggesting that the topographic gradient in gas exchange had markedly lessened. Both studies are consistent with the topographic perfusion model (West et al. 1964; see Sect. III.B) that relates blood flow to arterial and venous hydrostatic gradients in the presence of a uniform alveolar pressure. This model predicts uniform blood flow at O-G.

Capillary Distension

The apical regions of the lung will be subject to greater capillary recruitment at O-G, and the thoracic blood volume should be increased. Furthermore,

there is increasing evidence that left ventricular preload (and, thus, pulmonary venous pressure) increases at the onset of head-down tilt and O-G (see under Sect. V.B). Nixon et al. (1979) showed not only an increased left ventricular preload during the first 24 hr of 5-degree head-down tilt but also that the DLCO increased significantly, suggesting an increase in pulmonary capillary blood volume. In fact, there may well be more filling of the pulmonary capillaries for a given set of pulmonary arterial and pulmonary venous pressures in the absence of gravity. A small increase in pressure should equally affect all pulmonary capillaries in the absence of hydrostatic gradients.

Gas Exchange

The LearJet data of Michels and West (1978) suggest that topographic gradients in ventilation perfusion ratio (\dot{V}_A/\dot{Q}_c) should virtually disappear. However, it is possible that intraregional gas exchange may become compromised by increases in capillary pressure and transudation, especially under conditions of exercise. This possibility has been proposed for many years (Permutt, 1967), but it has not yet been tested. Cohen et al. (1971) noted a widening in A-a oxygen gradients during head-out immersion but, there, the major mechanism is almost certainly the low lung volume in the presence of increased dependent airways closure (Bondi et al., 1976).

Pleural Pressure

Pleural pressure rises considerably during head-out immersion: Arborelius et al. (1972) observed a rise of 5 ± 2 mmHg (7 ± 3 cmH₂O). This is associated with a fall in FRC of over 1 L (Bondi et al., 1976).

Agostoni (1985) estimates, on the basis of an extensive review, that at FRC, pleural pressure is about -6 mmHg (-8 cmH₂O) just below the lung apex, and -1.5 mmHg (-2 cmH₂O) at the lung base when sitting erect, and -3 mmHg (-4 cmH₂O) at the lung apex and 0 mmHg (0 cmH₂O) at the base in the supine posture. It is noteworthy that he considers that pleural pressure at the resting volume of the respiratory system is more subatmospheric at apex than at base in all postures. In space, if this still holds true, and if resting lung volumes are decreased, as they are in recumbency, basal pleural and, by inference, pericardial pressure may be increased. One hopes that this speculation about pleural pressure change at O-G is tested before too much is made of small measured changes in CVP or atrial pressure and that future CVP measurements in space (and during tilt simulation) are related, at least, to any changes in FRC or esophageal pressure.

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*Circulatory Changes**Volume Redistribution*

Simulations suggest that the cephalad fluid shift occurs in two phases: First, a shift of blood should occur and be complete within 20 to 30 sec (Sjöstrand, 1953). Arborelius et al. (1972) measured an abrupt increase in thoracic blood volume of 700 ml during head-out immersion. The erect-supine transition leads to a similar shift (Blomqvist and Stone, 1985). Second, there is a shift of extracellular fluid that can lead to a transient phase of plasma expansion. McCally et al. (1966) measured a 4.3% decrease in hematocrit 25 min after initiating immersion, which suggested a 9% increase in plasma volume. Hagen et al. (1978) saw a 440-ml expansion of plasma volume after 35 min in the supine posture.

The sum of the shifts observed in both immersion and bed rest can be seen to lie in the 1 to 2 L range.

Right Atrial Pressure: Right Ventricular Preload

Head-out immersion leads to a central venous pressure (CVP) increase, of about 12 torr above supine control values, that persists almost unchanged for 3 hr (Echt et al., 1974). On the other hand, head-down tilt studies showed smaller increases in CVP from supine control readings and Nixon et al. (1979) found that CVP was back to the supine baseline within 90 min of the onset of a 5° head-down tilt. Gaffney et al. (1985) showed a more prolonged CVP rise in a very similar study on older subjects. Katkov et al. (1979) saw no increase in right atrial pressure during a 15° head-down tilt study. Gauer and Thron (1965) found that the hydrostatic indifference point, normally just below the diaphragm, moves to a point just above the atria during head-down tilting. The fact that the right atrium is so close to this point makes the modest and variable effect of tilt understandable. These big differences between immersion and tilt studies pose the question: Is either model appropriate?

A prolonged CVP rise was seen in an orbiting macaque monkey (Meehan, 1971), and Soviet investigators have reported sustained jugular venous pressure increases (Levy and Talbot, 1983a). However, Kirsch et al. (1984), who estimated CVP in Spacelab-1 crewmembers from antecubital vein measurements, concluded that it had fallen. Unfortunately, the first inflight measurement opportunity was after 20 hr in orbit. Direct early measurements of CVP are planned during the Spacelab Life Sciences-1 (SLS-1) mission (West, 1984), and there is a wide consensus that further ground-based modeling is of very little value without this information (Levy and Talbot, 1983a, 1983b).

Pulmonary Arterial Pressure: Right Ventricular Afterload

Arborelius et al. (1972) measured pleural, pulmonary arterial (P_a), and right atrial pressure during head-out immersion. The pleural pressure rose 5 mmHg (FRC is reduced markedly during immersion), and the mean transmural P_a and right atrial pressures increased by about 13 torr.

Katkov et al. (1983) measured vascular pressures, but not pleural pressure, during a 7-day, 15° head-down tilt study. The mean P_a pressure rose slightly from 13.6 to 16.8 torr for the first 7 hr after transition from the supine control state. It is possible, however, that there was no transmural pressure rise: head-down tilting may reduce lung volumes, and thus, increase pleural pressure, and pleural pressure topography must also change.

It is widely recognized, however, that the absence of hydrostatic pressure gradients within the thorax at O-G may profoundly affect hemodynamics. Pulmonary vascular resistance should be lower for any given flow if the recruitment and distension of the capillary bed is topographically homogeneous. It is interesting that echocardiography, after 4 hr in orbit, showed decreased right ventricular end-systolic dimensions (Bungo et al., 1986 and personal communication).

Pulmonary Venous Pressure: Left Ventricular Preload

Left ventricular end-diastolic diameter seems to increase during the first 24 hr of head-down tilt, with an associated increase in stroke volume but a decrease in heart rate (Nixon et al., 1979). Pottier et al. (1986) have reported echocardiographic evidence for a 15% increase in left ventricular end-diastolic volume during the first 4 days of spaceflight. Recently, Bungo et al. (1986) found that while left ventricular end-diastolic volumes increased after 4 hr of microgravity, they were decreased below baseline on the second and subsequent days of flight.

Arterial Pressure: Left Ventricular Afterload

All Skylab crews had lower mean arterial pressures than those measured preflight (Johnson et al., 1977).

Cardiac Output

Head-out immersion can produce an over 30% increase of both cardiac output and stroke volume that shows no tendency to decrease after several hours (Arborelius et al., 1972; Begin et al., 1976).

Head-down tilt, in young male subjects, increased stroke volume but not cardiac output (Nixon et al., 1979). A later study of middle-aged subjects, by the same group (Gaffney et al., 1985), demonstrated an increase in both stroke volume and (for less than 4 hr) cardiac output. In other words, bradycardia was a significant mechanism in the younger but not in the older subjects. Despite the increase in cardiac output in the older subjects, arterial pressure was controlled by vasodilation. All hemodynamic

changes had returned to baseline in 20 hr in the tilt study of Gaffney et al. (1985), and they were smaller in magnitude than in the earlier immersion studies.

There is speculation that fluid shifts should lead to a rise in preload early in spaceflight, but whether or not the observation by Bungo et al. (1986) of an increased left ventricular end-diastolic volume is representative, and whether or not this is associated with an increase in cardiac output remains to be seen.

B. Adaptations to Microgravity

Negative Fluid Balance

Weight loss of 1 to 2 kg, approximately corresponding with the cephalad fluid shift, is seen within 3 to 4 days during bed rest and within 1 to 2 days during head-down tilt and space flight. Weight loss during water immersion is faster and can be more than 1 kg in 6 hr (Blomqvist and Stone, 1985).

Atriorenal Reflex

An atriorenal reflex (Gauer et al., 1970) that leads to an appropriate diuresis as central blood volume increases has a well-established role in the dog (Menninger, 1985). Dog low-pressure cardiac receptors are demonstrably more sensitive to small blood volume changes than are arterial baroreceptors (Gupta et al., 1966) and, through vagal afferents, tonically inhibit ADH release. Both of these low-pressure receptors and carotid sinus receptors also restrain the release of renin (Thames et al., 1978; Thames and Schmid, 1981). Cardiac denervation experiments attenuate the vasopressin response to hemorrhage (Wang et al., 1983) and grossly disrupt the relationship between osmolality, vasopressin, and blood volume (Wang et al., 1984).

Other Causes of Diuresis

In primates, there is a greater redundancy of mechanisms to defend the blood volume: cardiac denervation has either no effect on, or merely blunts the renal response to, volume loading (Peterson et al., 1983; Peterson and Jones, 1983; Cornish and Gilmore, 1982; Gilmore, 1983). Sino-aortic baroreceptors may play a more prominent role in vasopressin release (Menninger, 1985). There is also increasing awareness that atrial distension evokes other responses including an independent natriuresis which persists after vasopressin administration. Its rapid onset suggests that mechanisms other than aldosterone suppression are involved (Epstein et al., 1972), and a number of mechanisms, including the release of prostaglandin E and redistribution of renal bloodflow, have been suggested (Epstein, 1978). Atrial natriuretic factor may be involved, and this agent may also suppress renin release (Burnett et al., 1984).

Lack of Evidence for Diuresis in Spaceflight

The expected early diuresis was not observed during the Skylab program (Dietlein, 1977). Although ADH excretion levels were generally reduced during the Skylab program, there were some unexpected increases. These have been attributed to a negative fluid balance caused by such factors as decreased fluid intake and frank motion sickness (Leach and Johnson, 1985). Another increasingly recognized factor is the operationally necessary prolonged prelaunch recumbency: adaptation to this state may be well advanced at the time of transition to O-G. The O-G transition may not, therefore, represent a significant thoracic volume challenge.

Greenleaf (1985) speculates that atrial stretch occasioned by transition to O-G may be insufficient to trigger the rapid "Gauer-Henry" reflex, but may be sufficient to release natriuretic peptides and, thus, cause a slow secondary volume adjustment.

Circulatory Adaptations

Changes in Venous Compliance, Autonomic Changes

Yegorov (1979), using an occlusion plethysmographic technique, demonstrated a decrease in leg venous tone during Salyut-6-Soyuz flights. Echt et al. (1974) observed a rapid decrease in elasticity in arm veins at the onset of thermoneutral immersion, followed by a continuing decline in elasticity coefficient.

Large changes in venous tone can have very dissimilar effects on blood distribution in different gravitational orientations and states. Head-out immersion increases the pressure gradient across the diaphragm (Hong et al., 1969). Under these circumstances, a relaxation of splanchnic and peripheral venous tone must have little effect on the blood shift to the intrathoracic compartment. The same argument could be applied to supine head-down tilt; it seems intuitively obvious that in the extreme event of relaxed inversion, the major caudal capacitance vessels will remain collapsed, no matter what the state of venoconstriction. On the other hand, extravascular pressures may be sufficiently uniform during weightlessness for venous unstressed volume and capacitance changes to substantially alter the distribution of blood between the intrathoracic and other compartments, thus, decreasing any major initial increase in intrathoracic blood volume.

Cardiac Effects

It is known that the circulatory blood volume is reduced after spaceflight (Blomqvist and Stone, 1985; Johnson et al., 1977). There is recent evidence that cardiac volumes are reduced below baseline supine values within 4 days of microgravity exposure (Bungo et al., 1986). Henry et al. (1977) found a similar reduction in cardiac volumes and a small decrease in

estimated left ventricular mass in two of three subjects after prolonged spaceflight, but found no change in intrinsic cardiac performance. Soviet (Rokhlenko and Mul'Diyarov, 1981) and American (Philpott et al., 1985) investigators have both reported ultrastructural myocardial abnormalities in rats exposed to short-term microgravity.

C. Postflight Effects

Orthostatic Intolerance

Postflight faintness on standing is common. Passive standing, 70° head-up tilting (Fig. 8), and lower-body negative pressure (LBNP) have consistently led to more disturbance postflight than preflight. Pulse rates are higher,

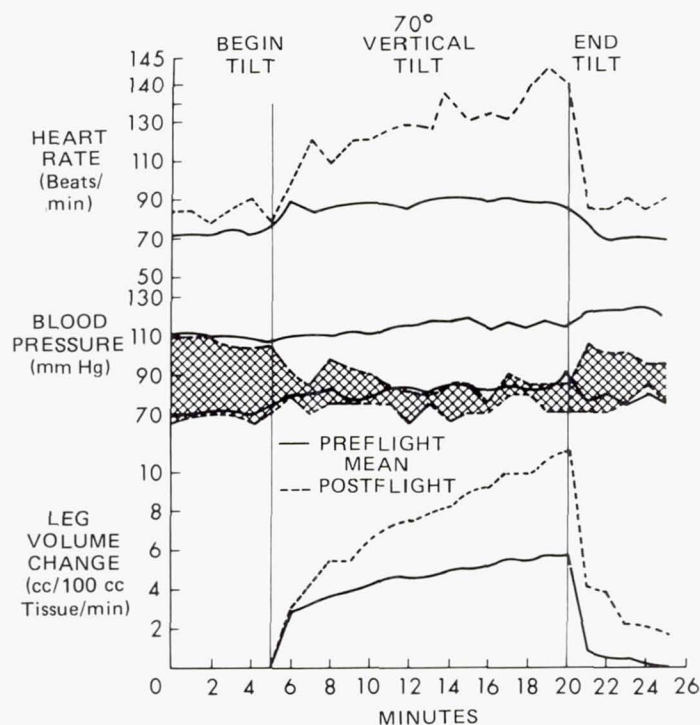


Figure 8 Tilt-table data from one subject before and after spaceflight (Gemini 7). Note the larger postflight (dashed and hatched) effects on heart rate and blood pressure and the apparent increase in leg volume change. The Whitney strain gauge measurement of leg volume change is of doubtful validity—see text (from Dietlein, 1977, with permission).

systolic blood pressures are lower, pulse pressures are narrower, and cardiac outputs are decreased (Sandler, 1980). This was initially attributed to an increase in venous pooling, as evidenced by Whitney strain gauge and capacitance band measurements of leg volume (see Fig. 8) (Johnston et al., 1977; Thornton and Hoffler, 1977), but this observation is suspect. Both of the preceding techniques detect relative volume changes, rather than absolute volume values, and are sensitive to the decrease in leg volume that occurs in flight and in simulations. Musgrave and coworkers (1969), who used a water plethysmographic technique, showed no increase in pooling after bed rest. There is a 50- to 60-ml decrease in left ventricular end-diastolic volume after 50 torr LBNP, both before and after bed rest, despite a marked increase in the other indicators of orthostasis (Sandler, 1980; Sandler et al., 1977).

After Skylab missions, left ventricular end-diastolic volume was significantly decreased (Henry et al., 1977), as was blood volume (Johnson et al., 1977). Tissue pressure falls in normally dependent regions during adaptation to head-down tilt (Hargens et al., 1983). This presumably also occurs in weightlessness and sets the stage for a further decrease in blood volume when hydrostatic gradients are restored, a result of rapid reestablishment of normal tissue volumes and pressures by transudation. Despite the ingestion of 1 L of saline before reentry, postflight echocardiograms of shuttle crew members have shown low end-diastolic volumes and stroke volumes (Bungo et al., 1987). Hypovolemia and left ventricular volume reduction set the stage for an increased sensitivity to similar amounts of pooling.

Vasovagal Fainting

Epstein and associates (1968) have described the sequence of events that occur in normal subjects on tilting or exposure to LBNP. Pooling decreases left ventricular preload and tends to lower blood pressure. If this occurs, the reduction in left ventricular afterload should lead to a gross reduction in left ventricular end-systolic volume, which may stimulate intracardiac receptors causing bradycardia, vasodilation in muscles, and collapse. After spaceflight, when left ventricular volumes are already reduced, orthostasis can readily lead to fainting.

To avoid orthostatic collapse, a brisk autonomic response is required. However, baroreceptor stimulus response curves may alter during spaceflight in the absence of normal inputs, and decreased sympathetic traffic to the peripheral vasculature may cause depletion of the endogenous catecholamine stores. Schmid et al. (1971) and Steinberg (1980) demonstrated that norepinephrine infusion after bed rest produced a normal increase in venous tone, but tyramine (which releases endogenous catecholamines) did not.

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Many mechanisms may, therefore, contribute to postflight "cardiovascular deconditioning." The latter term is unfortunate if applied to the total postflight syndrome, because there are almost certainly muscular and neural adaptations to microgravity that contribute to the weakness and diminished exercise tolerance observed postflight.

*Artificial Countermeasures to Adverse Effects**Reducing Inflight Adaptation*

Greenleaf (1984) and Blomqvist and Stone (1985) have reviewed the countermeasures that have been proposed to improve orthostatic tolerance and point out that the flight environment is not only hypogravic, but it is also hypodynamic: little exertion or movement is required in space. Exercise of leg muscles during bed rest can decrease orthostatic intolerance, whereas continuing gravitational stress (periodic standing, chair sitting) without exercise is ineffective. More pronounced stress (LBNP, +G_z) can be an effective countermeasure without exercise. Exercise has become a routine countermeasure on all spaceflights, but this has not eliminated orthostatic intolerance (Greenleaf et al., 1975; Johnson et al., 1977; Kakurin et al., 1980; Sandler, 1980).

The Soviets use volume-redistribution devices inflight as a means of reducing adaptation. The "Chibis" vacuum suit, a variant of a lower-body negative pressure device, is used towards the end of a mission, once every 4 days for 20 min and, then, 50 min a day for the last 2 days (Gazenko et al., 1981; Nicogossian and Parker, 1982).

Decreasing Postflight Effects

Water and salt loading just before reentry has been shown to reduce the postflight orthostatic fall in blood pressure otherwise observed in shuttle crews (Bungo et al., 1985). Fluid loading (ingestion of approximately 1 L of water plus 8 g of salt tablets) before reentry is now required as a means of reducing postflight orthostatic intolerance in shuttle crews. Unfortunately, this requirement tends to confound further investigation.

G-suits are available to shuttle crews for use during reentry when up to +2G_z can be experienced (a level that may have a marked effect on a deconditioned subject). Their use is, however, at the discretion of the wearer. G-suits and pressure-gradient elastic leotards have also been used postflight with considerable efficacy (Buyanov et al., 1967; McCally et al., 1968; McCally and Wunder, 1971; Miller et al., 1964).

VI. Conclusions

Aviators tolerate pressure breathing (PB) remarkably well for the short periods used operationally. This may be the reason for the very few

published reports of its effects. There has long been an appreciation that left ventricular afterload is reduced during PB, despite elevation of arterial pressure. It has been assumed that diminished venous return is entirely responsible for decrease in cardiac output. There has been little appreciation of the effects of PB on right ventricular function, probably because the best studies have been performed with trunk counterpressure limiting lung expansion. It is obviously time to reexamine this topic, in the light of the findings during modest PB in relaxed humans and animals, and especially because trunk counterpressure is not used in most aviation applications, including protection against the effects of $+G_z$.

It seems that resisted PB leads to much less reduction in cardiac output than relaxed PB. Some of this difference may be due to lowering of lung volume, but much must be due to a greater shift of the venous return curve to the right. There is obviously considerable utility in any adaptation that balances a rise in intrathoracic pressure with a rise in mean systemic pressure. Straining would seem to do this by inducing concurrent increases in abdominal muscle tone and venous tone. These mechanisms probably evolved to protect the circulation during vigorous straining with a closed glottis (for example, during heavy lifting in humans).

There are obvious parallels between the mechanisms affording protection during straining with a closed glottis, and the mechanisms that extend $+G_z$ tolerance. Pigs illustrate this on the centrifuge when they alternately strain with a closed glottis (which raises eye-level arterial pressure), then, briefly relax (which allows venous return), in an optimum repetitive sequence and, thereby, extend their $+G_z$ tolerance.

Centrifuge studies may reveal other heart-lung interactions. For example, basal pleural pressures can be elevated and the lung compressed by $+G_z$ with G-suit inflation, and there is some evidence that even elevated atrial pressures do not restore cardiac output. These observations could obviously be related, but coordinated measurements of intracardiac and extracardiac pressures have not yet been made.

There has been speculation that major heart-lung interactions should occur in microgravity. A "working hypothesis" was developed after Skylab that a headward redistribution of blood and extracellular fluid should initially expand the intrathoracic vascular compartment. This should lead to a number of adaptations including diuresis which, in turn, might lead to, or at least augment, the orthostatic intolerance seen after flight. We wish we were able to review a coordinated investigation that had tested this hypothesis. The Spacelab Life Sciences-1 Mission includes several human investigations that should go a long way toward this goal. Serial measurements will be made during the 7-day flight, and both preflight and postflight. Central venous pressures and echocardiographic

volumes will be a major part of a cardiology investigation. Estimations of pulmonary blood flow and tissue volume will be made both at rest and during exercise, and capillary volume estimations will be made at rest. A comparison of the time course of changes of these variables with changes in body mass, urine flow, and endocrine status may allow future reviewers to be less speculative.

Abbreviations and Symbols

A-a gradient:	alveolar-arterial O ₂ gradient
ADH:	antidiuretic hormone
CVP:	central venous pressure
DLCO:	diffusing capacity of the lung for carbon monoxide
ERV:	expiratory reserve volume
FI _{O₂} :	inspired fraction of O ₂
FRC:	Functional residual capacity
G:	acceleration normalized to gravitational acceleration (a/g)
+G _x :	increased back-to-front acceleration (eyeballs-in)
+G _y :	increased left-to-right acceleration (eyeballs-left)
+G _z :	increased foot-to-head acceleration (eyeballs-down)
LBNP:	lower-body negative pressure
LVEDP:	left ventricular end diastolic pressure
MAST:	medical antishock trousers
M-1:	the M-1 anti-G straining maneuver
P _A :	alveolar pressure
PACO ₂ :	alveolar partial pressure of CO ₂
PAO ₂ :	alveolar partial pressure of O ₂
P _a :	pulmonary arterial pressure
PaO ₂ :	arterial partial pressure of O ₂
PB:	pressure breathing
PEEP:	positive end-expiratory pressure
P _v :	pulmonary venous pressure
TLC:	total lung capacity
TSR:	total systemic resistance
\dot{V}_A/\dot{Q}_c :	ventilation/perfusion ratio
O-G:	weightlessness (in reality: microgravity)

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THREE HIERARCHIES IN SKELETAL MUSCLE FIBRE CLASSIFICATION
ALLOTYPE, ISOTYPE AND PHENOTYPE¹

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ABSTRACT Immunocytochemical analyses using specific anti-myosin antibodies of mammalian muscle fibres during regeneration, development and after denervation have revealed two distinct myogenic components determining fibre phenotype. The jaw-closing muscles of the cat contain superfast fibres which express a unique myosin not found in limb muscles. When superfast muscle is transplanted into a limb muscle bed, regenerating myotubes synthesize superfast myosin independent of innervation. Reinnervation by the nerve to a fast muscle leads to the expression of superfast and not fast myosin, while reinnervation by the nerve to a slow muscle leads to the expression of a slow myosin. When limb muscle is transplanted into the jaw muscle bed, only limb myosins are synthesized. Thus jaw and limb muscles belong to distinct allotypes, each with a unique range of phenotypic options, the expression of which may be modulated by the nerve. Primary and secondary myotubes in developing jaw and limb muscles are observed to belong to different categories characterized by different patterns of myosin gene expression. By taking into consideration the pattern of myosins synthesized and the changes in fibre size after denervation, 3 types of primary (fast, slow and intermediate) fibres and two types of secondary (fast and slow) fibres can be distinguished in rat fast limb muscles. All primaries synthesize slow myosin soon after their formation, but this is

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withdrawn in fast and intermediate primaries at different times. After neonatal denervation, slow and intermediate primaries express slow myosin whereas fast primaries do not, and slow primaries hypertrophy while other fibres atrophy. In the mature rat, the number of slow fibres in the EDL is less than the number of slow primaries. Upon denervation, hypertrophic slow fibres matching the number and topographic distribution of slow primaries appear, suggesting that a subpopulation of slow primaries acquire the fast phenotype during adult life, but reveal their original identity as slow primaries in response to denervation by hypertrophying and synthesizing slow myosin. It is proposed that within each muscle allotype, the various isotypes of primary and secondary fibres are myogenically determined, and are derived from different lineages of myoblasts.

INTRODUCTION

Fibres of limb and trunk muscles of mammals have been classified phenotypically into slow, fast-red and fast-white types, each type containing a distinct type of myosin, and associated with a specific profile of metabolic enzymes. Consequently, these various types of fibres differ in intrinsic speed of contraction, power output and endurance. Such phenotypic diversity has been attributed to the myoregulatory function of the motor nerve supply. According to this hypothesis, muscle fibres are considered to be "plastic", and fibre types interconvertible according to the pattern of impulses received from the nerve (1,2).

During myogenesis, myotubes are uniformly slow contracting and synthesize embryonic and foetal myosins before expressing adult fibre characteristics. In view of the profound influence motor nerves have on mature muscle fibres, it has generally been considered, since the work of Buller, Eccles and Eccles (3,4) that the emergence of muscle fibre heterogeneity during myogenesis is also brought about by the action of nerves on a common, undifferentiated myotube.

The experiments described in this paper were done to test the neural regulatory hypothesis for fibre type diversity. The jaw muscles of carnivores contain superfast muscle fibres which express a unique myosin not found in limb muscles. If the neural regulatory hypothesis is valid for these muscles, jaw muscles regenerating in limb muscle

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beds should express limb muscle myosins and vice versa. During limb muscle development, muscle fibres are poly-neuronally innervated. Emerging myotubes would be expected to co-express a mixture of adult myosins. The results of these experiments obtained with immunocytochemical techniques using monoclonal and polyclonal anti-myosin heavy chain antibodies do not confirm these expectations. They reveal two hierarchically distinct levels of myogenic influences affecting fibre phenotype. A hierarchical classification is proposed in which jaw and limb muscles belong to different allotypes which define their phenotypic options. Within each allotype, myogenically distinct isotypes emerge during development.

RESULTS

Nerve Independent Intrinsic Differences Between Cat Jaw and Limb Muscles

Strips of posterior temporalis muscle, a homogeneous superfast muscle, were treated with Marcaine to destroy mature muscle fibres and transplanted into limb muscle beds for regeneration and reinnervation by the host nerve (5). Early regenerates in the bed of either the fast extensor digitorum longus (EDL) or the slow soleus muscle react with antibodies against the heavy chain of foetal, slow or superfast myosins, but not with antibodies against fast myosin. In the long-term, regenerates innervated by the EDL nerve express only superfast myosin whereas in the regenerates innervated by the soleus nerve most fibres react only with the anti-slow myosin antibody, while some fibres react only against superfast myosin even after 213 days. In contrast, EDL and soleus muscles regenerating in their own beds express foetal, slow and fast myosins, but do not express superfast myosin. The isometric contraction times of the various types of regenerates reflect the types of myosin synthesized.

The ability of the regenerating superfast muscle to express the superfast myosin is independent of the nerve (6). This is shown in experiments in which reinnervation of the transplant in the EDL bed is prevented by cutting the common peroneal nerve and reflecting it back into the thigh. In these denervated beds the early temporalis regenerates are indistinguishable from innervated regenerates in expressing superfast myosin in addition to foetal and slow myosins.

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have also been shown by the transplantation of limb muscles into the jaw muscle bed. Jaw muscle beds are less satisfactory from the point of view of defining the innervation of the regenerates. Limb muscle strips were transplanted into the anterior temporalis bed after partial excision of this muscle. Fig. 1 shows the results of an EDL regenerated in the anterior temporalis muscle bed for 12 weeks. Staining for superfast (Fig. 1a) and foetal (Fig. 1b) myosins is negative, whereas nearly all fibres stain for fast myosin (Fig. 1d), and some fibres also stain for slow myosin (Fig. 1c). Although innervation of these fibres is not specifically demonstrated, their large size and the absence of staining for foetal myosin suggests that they are innervated.

It is concluded that jaw and limb muscle cells are two distinct types of muscle cells, each having a distinct repertoire for the expression of adult isomyosins, and that the particular isomyosin expressed can be modulated by the nerve.

Heterogeneity of Primary Fibres in Developing Limb Muscles

The postnatal development and the effects of neonatal denervation on muscle fibres in the EDL and tibialis anterior (TA) muscles of the rat were studied immunocytochemically using monoclonal antibodies against myosin heavy chains. Three types of primary myotubes (fast, intermediate and slow) with distinct topographic distributions can be distinguished perinatally. All primaries synthesize slow myosin initially, but in fast and intermediate primaries, slow myosin is no longer detectable in the neonatal period and at 2 weeks of age respectively. The fast primaries are localized principally in a superficial strip of the TA (Fig. 2B) where in the matured muscle slow fibres are absent. Slow primaries are located deep in the muscle while intermediate primaries lie in between. The distribution of slow and intermediate primaries at birth is shown in Fig. 2A.

Following neonatal denervation, the slow and intermediate primaries still express slow myosin, whereas the fast primaries do not stain for slow myosin. At three weeks after denervation, slow primaries are hypertrophic and intermediate primaries are atrophic, both staining with anti-slow antibody. The topographic distribution of these primaries is shown in Fig. 2C. These results show that the three different types of primary myotubes respond

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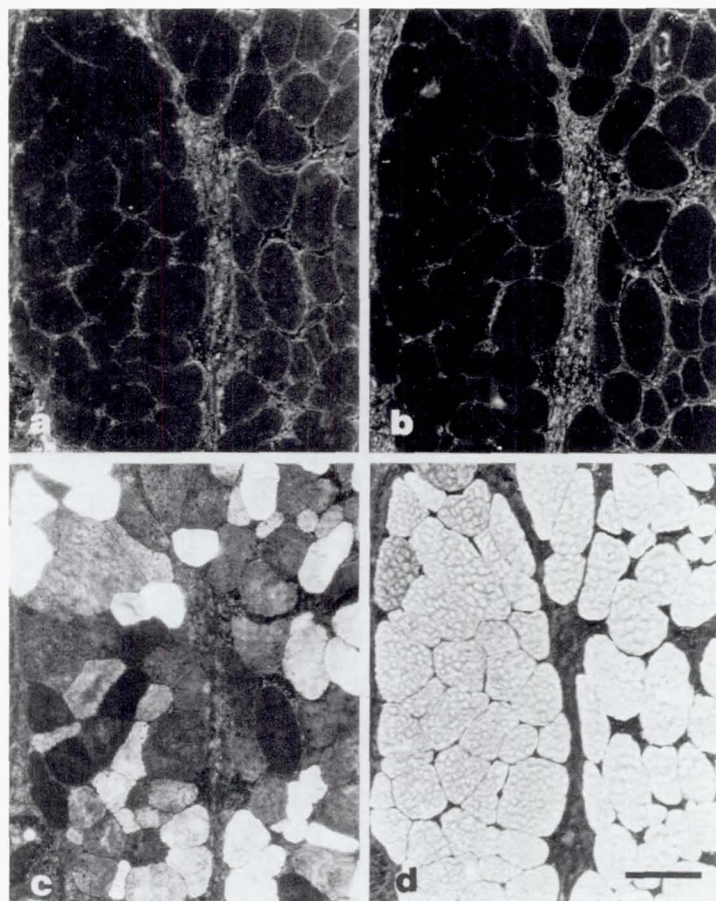


Fig. 1. Fluorescence photomicrographs of serial sections of cat extensor digitorum longus muscle regenerated in the anterior temporalis muscle bed for 12 weeks stained for superfast (a), foetal (b), slow (c), and fast/foetal (d) myosin heavy chains. The scale represents 100 μ m.

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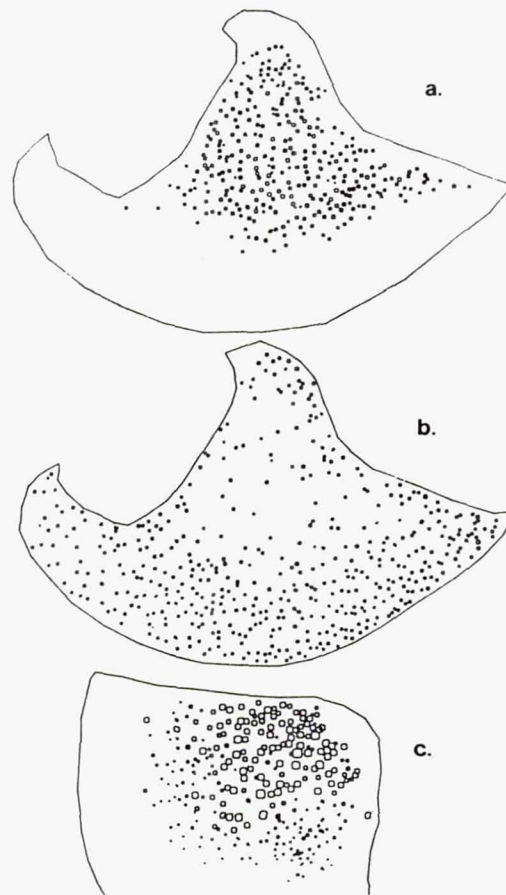


Fig. 2. Distribution of fibres which stain with anti-slow myosin heavy chain antibody in rat tibialis anterior (TA) muscle at birth (A, B) and TA three weeks after neonatal denervation (C). Fibres which stained strongly (slow and intermediate primaries) and those that stained faintly in the neonatal TA (fast primaries) are shown in (A) and (B) respectively. Note the hypertrophy of slow primaries and atrophy of intermediate primaries following denervation (C).

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differently to denervation.

Heterogeneity of Secondary Fibres in Developing Limb Muscles

Immunocytochemical analysis of neonatal cat EDL and soleus muscles (7) has revealed that there are at least two distinct classes of secondary fibres. Both classes initially stain strongly for embryonic/foetal myosins. In one of these classes, developmental myosins are replaced by fast myosin. These fast secondaries do not stain for slow myosin nor react with anti-superfast myosin antibody at any stage. The other class of secondaries, the slow secondaries, are prevalent in the slow soleus muscle. These fibres acquired staining for slow myosin but not for fast or superfast myosins.

The vast majority of neonatal secondary myotubes in rat EDL and TA muscles are fast secondaries and stain with an anti-foetal/fast-red myosin antibody. These myotubes diverge at 9 days into a superficial fast-white region and a deep fast-red region. The majority of superficial secondaries no longer stain for foetal/fast-red myosins, presumably expressing fast-white myosin, whereas secondaries located in the region occupied by slow primaries predominantly express fast-red myosin. This topographical distribution of the two classes of secondaries is present in both the EDL and TA muscles, but is more conspicuous in the latter.

Following neonatal denervation in the rat, the divergence of fast-red and fast-white fibres in the EDL and TA muscles is not abolished, but delayed till three weeks post-operatively, suggesting that this divergence is neurally independent.

Effects of Denervation on Slow Primaries in Adult Rats

Immunocytochemical analyses of rat limb skeletal muscle fibres using specific anti-myosin antibodies have revealed that post-denervation changes of muscle fibres cannot be predicted by the fibre phenotype (8). The number of slow fibres in the EDL of a three month old rat is about half the number of slow primaries seen during development. Upon denervation of this muscle, the number of slow fibres increases to match the number of slow primaries at birth. These fibres hypertrophy while other fibres suffer denervation atrophy. These observations suggest that about half

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of the slow primaries in mature rats undergo fibre type transformation into phenotypically fast fibres. Upon denervation, all slow primaries express slow myosin and hypertrophy, just as they do after neonatal denervation, irrespective of their phenotype at the time of denervation.

Heterogeneity of Primary and Secondary Fibres in Developing Cat Jaw Muscles

There are two phenotypes in jaw-closing muscle fibres in the cat: superfast and slow. In the posterior temporalis muscle of the mature animal, all fibres are superfast. During late foetal life, sections of this muscle stained for slow myosin appear very similar to those of fast limb muscle: slow staining primary fibres surrounded by rosettes of secondary fibres. Later, both primary and secondary fibres synthesize superfast myosin and the slow myosin in primary fibres is withdrawn (9). Primary fibres in the posterior temporalis are therefore analogous to fast primaries in limb muscles, and may be termed superfast primaries. Slow fibres are present in the anterior temporalis and the masseter muscles of adult cats, and these developmentally are derived from both primary and secondary fibres. The jaw slow primary fibres are analogous to slow primaries in limb muscles in which slow myosin synthesis persists to adult life. The jaw slow secondaries appear in early postnatal life, and some of these fibres stain also for superfast myosin during this period. At no time during the development of cat jaw muscle fibres does any fibre stain for fast myosin.

DISCUSSION

The results of these experiments reveal that the neural regulatory hypothesis cannot account for the difference between limb and jaw muscles. Each type of muscle has a specific repertoire for myosin gene expression, the limb muscles express slow, fast-red and fast-white myosins while jaw muscles express slow and superfast myosins. The ability to express these myosins is intrinsic to the muscle type, and can occur during regeneration in the absence of innervation. Innervation by limb nerves does not induce jaw regenerates to express fast myosins, nor does innervation by jaw nerve fibres lead to the expression of superfast myosin in limb regenerates. However, the specific form of myosin expressed by jaw or limb muscles is subject

to neural regulation within the constraints of the repertoire. The limited repertoires for myosin gene expression for jaw and limb muscles is also seen during developmental myogenesis.

It is useful to introduce the term allotype to describe different classes of skeletal muscle fibres with distinct intrinsic properties such as limb and jaw muscles. Jaw and limb allotypes probably arise from distinct lineages of myoblasts committed to differentiate along different paths. Extraocular muscles, which are isometrically faster than limb and jaw muscles (10) and which express a unique myosin heavy chain (11) may be another skeletal muscle allotype.

Immunocytochemical analyses of developing limb and jaw muscles reveal considerable heterogeneity in the pattern of myosin gene expression in both primary and secondary fibres. Such heterogeneity may be due to some extrinsic influence, such as innervation, acting upon a homogeneous population of myotubes. Alternatively, the myotubes may be intrinsically heterogeneous, being preprogrammed to express different types of myosin during subsequent development.

Evidence against the suggestion that fibre type diversity emerges as a result of neural regulation is the observation that divergence of fast and slow primaries is already apparent prenatally (12) whereas the impulse patterns of developing fast and slow motoneurons in the neonatal rat are very similar; differences emerge only at 3 weeks postnatally (13). Furthermore, the occurrence of polyneuronal innervation of muscle fibres (14) in the early postnatal period also argues against the neural regulatory hypothesis.

In support of the notion that myotubes are intrinsically heterogeneous may be cited the observations that clonal colonies of early myoblasts in chicken (15) and human embryos (16) are not homogeneous with respect to nutrient requirements and colony morphology. Miller and Stockdale (17) have isolated three types of clones from early chicken myoblasts which express fast, slow or a mixture of both myosins. These clones provide a nerve-independent mechanism for the generation of different muscle fibre types during myogenesis (18).

We propose that the emergence of diverse primary fibres in mammalian limb and jaw muscles is due to various lineages of myoblasts with intrinsically different properties. The characteristic responses to neonatal denervation of the three types of primaries in the rat limb clearly reveal their differences, the most spectacular feature of which being the

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hypertrophy of slow primaries. This property of slow primaries is retained in the adult even though some of the slow primaries had apparently undergone, through the neural regulatory influence, a phenotypic change to become fast fibres. Thus, the hypertrophic response of denervated adult muscle fibres cannot be predicted on the basis of fibre phenotype, but can be so predicted according to the developmental origin of the fibres. Hence it is important to classify muscle fibres in accordance with their developmental origin in addition to their allotype and their phenotype. We propose to use the term isotype in this context. Thus there are at least three isotypes (slow, intermediate and fast) of primary fibres in limb muscles and two isotypes (superfast and slow) of primary fibres in jaw muscle.

The emergence of phenotypic characteristics of secondary fibres occurs relatively late during myogenesis, making it possible for neural regulatory mechanisms to have an impact on it. However, the possibility of there being various isotypes of secondary fibres cannot be discounted. Our neonatal denervation study shows that the divergence of fast-red and fast-white fibres is neurally independent, raising the possibility of the existence of two distinct isotypes of fast secondary myotubes. An interesting alternative mechanism for generating different phenotypes of secondary fibres is for primary fibres to influence the phenotype of the secondary fibres associated with them. The existence of gap junctions between primary and secondary fibres is well established (19). These junctions may provide the physical basis for the postulated myogenic influence on secondary fibres. The co-localization of slow primaries and fast-red fibres in the deep region of TA is consistent with the notion that slow primaries induce the expression of the fast-red phenotype in secondary myotubes associated with them.

The various myogenic and neurogenic influences on the phenotypic expression of myosin genes during myogenesis may now be summarized. The allotype defines the various phenotypic options available: superfast and slow myosins for the jaw allotype and fast-red, fast-white and slow for the limb allotype. Very early in myogenesis, diverse isomyoblasts emerge within each allotype. These fuse to produce myotubes of corresponding isotypes, each destined to undergo a particular pattern of myosin gene expression within the options defined by the allotype. Innervation may only play a trophic or permissive role on myogenesis up to this point.

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Neural regulatory influences may operate after polyneuronal innervation has been eliminated and phasic and tonic nerve impulse patterns established. These influences may change the fibre phenotype within the range of options defined by the allotype, but do not alter the fibre isotype, nor transform the fibre allotype.

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COMPARATIVE ASPECTS OF HEMATOLOGICAL RESPONSES IN ANIMAL AND HUMAN MODELS IN SIMULATIONS OF WEIGHTLESSNESS AND SPACE FLIGHT

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This paper reviews some human and animal responses to space flight as well as in control models in simulations of weightlessness. Astronauts after space flight have been found to have a decreased red blood cell mass and plasma volume. The reason for these changes is unknown but appears to be caused primarily by a decrease in the need of red blood cells in the weightless condition. Similar though more moderate changes have been found in human subjects subjected to prolonged bed rest or water immersion. What happens to the red cell mass of laboratory rats flown in microgravity is not known but rats have shown an increase in the rate of random red cell loss in flight suggesting a probable decrease. Rat models subjected to either head-down suspension or restraint alone have shown a decrease in red blood cell masses and a decrease in their plasma volume.

I. Introduction

Numerous studies have shown that astronauts after flights have a reduction in their ⁵¹Cr red cell mass and ¹²⁵I HSA plasma volume and consequently a decreased blood volume (6,26). Similar changes have occurred in human subjects in simulations of weightlessness produced by bed rest with or without head-down tilt (1,8,10,13, 14,17,19,24). Far fewer studies have been carried out on animals flown in microgravity and it is not known whether the laboratory rat is a valid model for the changes which occur in humans during space flight (4,5,9,11,12,15,16,21-23). However, rats subjected to either antiorthostatic or orthostatic hypokinesia/hypodynamia exhibit some of the same changes in red cell mass and plasma volume found in astronauts after space flight (2,3).

We have compared the hematological changes found after space flight with changes found in simulated weightlessness. Because of limitations in space, we concentrate on the studies of human astronauts and their simulated controls on Spacelab 1 (SL-1). The focus on animal studies was on the results from animals flown on Spacelab 3 (SL-3) together with ground-based simulation experiments. The results of these studies have been previously published separately (2,3,11,12, 14). Results of other investigators will be discussed as space permits.

II. Human Studies

Although some of the red blood cell changes found in early flights of NASA spacecraft were undoubtedly due to hyperoxic damage to red blood cells caused by the utilization of an increased partial pressure of oxygen, the Russian experience plus the results of Skylab and shuttle studies effectively rule out hyperoxia as a cause of the decrease in red cell mass (6,26).

The results of Skylab reticulocyte studies pointed towards a decreased production of red blood cells. This was investigated in personnel who flew on SL-1 and in simulation subjects who were selected on the basis of similarity of age, weight, sex (male), physical condition and overall health status (14). During the simulation of the inflight period, the control subjects were placed at -6° head-down bed rest for a period equal to the flight period. The following table shows the changes in red cell mass and plasma volume in SL-1 flight personnel, and control subjects.

Table 1. Percent decrease in red cell mass (RCM) and plasma volume (PV)

	RCM		PV		N
	Mean	S.E.	Mean	S.E.	
SL-1, 10 days	9.3*	1.6	6.0	4.3	4
Bedrest, 10 days	4.6*	1.2	5.4	4.4	5

*Significantly ($p < .05$) different from preflight measurement.

The changes in reticulocyte numbers are shown in Table 2.

Table 2. Reticulocyte numbers $\times 10^9/L$

	Preflight	MD-1	L+0	L+8
Flight	64 \pm 5	49 \pm 15	24 \pm 8*	48 \pm 5
Bedrest	35 \pm 6	36 \pm 4	38 \pm 6	32 \pm 9

*Significantly different ($p < .05$) from preflight measurement. MD = mission day; L = landing day.

As shown in Table 2 the reticulocyte number decreased in the astronauts, indicating a probable decrease in production of red blood cells. However, this was certainly not complete and as shown in Table 3 incorporation of radioactive iron injected preflight was quite similar in control and flight subjects. The post-flight decrease in the calculated red blood cell iron incorporation suggests an increased red blood cell production in crew members.

Table 3. RBC iron incorporation SL-1 and bedrest simulation (% in RBC)

	MD-1	MD-7	L+0	L+1	L+8	L+12
Flight	19	85	88	93	85	86
Bedrest	21	86	91	92	91	92

MD = mission day; L = landing day.

Also mitigating a complete shutdown of bone marrow production is the fact that the levels of serum iron and iron-binding capacity were unchanged. This also shows that iron stores are replete. Serum ferritin is a measure of iron stores and on SL-1, as shown in Table 4, there were significant increases seen on MD-7, L+0, and L+1. This could indicate that the iron from red blood cells lost early in flight was being

reutilized and also that the inflight loss of red blood cells was not the result of external hemorrhage.

Table 4. Ferritin changes SL-1 and bedrest simulation (percent change).

	MD-1	MD-7	L+0	L+1	L+8	L+12
SL-1	1.5	53*	62*	63*	1.9	-15
Bedrest	1.3	-13.9	-11.6	-11.9	-34.2*	-33.0*

*Significantly different ($p < .05$) from preflight measurements. MD = mission day; L = landing day.

The other major cause for a loss of red blood cell mass would be increased red blood cell destruction. However, radioactive tracer studies on SL-1 crew members showed that the percentage of red blood cells remaining at 8 days was the same as it was for simulation subjects and was normal. Intravascular hemolysis should lead to decreased serum haptoglobin, but haptoglobin increased slightly but not significantly.

III. Animal Studies

Simulation studies for SL-3 flight animal studies were carried out using rats suspended in a jacket and harness arrangement. In the first study the head-down angle was approximately 20° and by use of fore limbs the rats were able to move through 360° (2).

The results in the suspended rats showed:

- Reduction in red blood cell mass,
- Suppression of erythropoiesis,
- A transient increase in hematocrit due to a reduction in plasma volume,
- A post-exposure hematocrit decrease,
- A weight loss (or failure to thrive),
- A reduction in food and water consumption.

Similar results are observed in man so at least in a gross sense, the rat "model" seemed to reproduce many of the known hematological effects found during and after space flight.

The studies were expanded to evaluate the effect of restraint alone as opposed to head-down tilt and many of the same changes were found (3). Changes in red blood cell clearance were thought to be unique to the head-down posture. This is currently being reevaluated and preliminary results have shown no change in red blood cells remaining in the circulating red blood cell mass. Thus, survival was normal (R. Nachman, unpublished observations).

While the changes in red cell parameters have been conclusively shown in astronauts, to our knowledge no isotope studies of red cell mass have been performed on rats flown in space so it is not known if the rat is indeed a potential model for "space anemia."

On SL-3, 24 rats were flown on the 8-day flight. The hematology studies performed after the flight showed (11,12):

- Hematocrits, red blood cell counts, and hemoglobin determinations were increased in flight animals. The number of reticulocytes were slightly decreased in the large rats and slightly increased in small rats but the differences were not significant.

- There were no significant differences from control animals in spleen cell differentials or erythropoietin determinations for control and flight animals.

- The bone marrow cells of flight animals demonstrated an increased sensitivity to erythropoietin when grown in methyl-cellulose cultures.

IV. Discussion

A comparison of the results of changes in red blood cell parameters in the human and rat studies are shown in Tables 5 and 6.

Table 5. Human studies

	SL-1			Bedrest Simulation 6° Head Down		
	L+0	L+8	L+13	L+0	L+8	L+13
RCM	↓*	↓	--	↓*	↓*	--
Plasma Vol	↓	↑	--	↓	NC	--
Blood Vol	↓*	NC	--	↓	↓	--
HCT	↓	↓*	↓*	↑	↓	--
RBCC	↓	↓*	↓*	↑	↓	↓
Hgb	↑*	↓*	↓*	↑	↓	↓
MCV	↑	↑	↑	NC	NC	NC
MCH	↑*	NC	NC	NC	NC	NC
MCHC	↑*	↓	↓	↑	↑	↑*
RBC Shape						
Discocyte %	↓	↓	NC	--	--	--
Echinocyte %	↑	↑*	↑	--	--	--
BM Ery.	--	--	--	--	--	--
Retics	↓	--	NC	↑	↓	↑
Fe Inc.	88%	--	--	91%	--	--
Serum Fe	NC	↓	↓	↓	↓	↓
Ferritin	↑*	NC	↓	↓	↓*	↓*
Haptoglobin	↑	↑	↑	↑	NC	NC
Epo	↓	↓	↓	↑	NC	NC
RBC Surv.	NC	--	--	NC	--	--

NC = Unchanged

* = Sig. < 0.05 .

Table 6. Rat studies

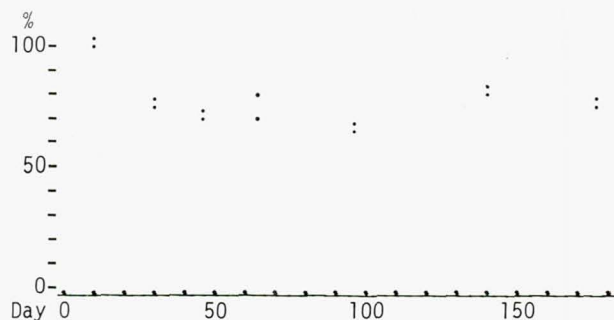
	SL-3		Head Down Suspended Rats		
	L+0		L+0	L+8	L+28
	Sm. Rats	Lg. Rats			
RCM	--	--	↓*	↓	NC
Plasma Vol	--	--	↓*	↓	NC
Blood Vol	--	--	↓*	↓*	NC
Hct	↑*	↑	↓*	↓*	↑
RBCC	↑*	↑	NC	↓	--
Hgb	↑*	↑	↓*	↓	--
MCV	↓	↓	↓*	↓	
MCH	↓	NC	--	--	--
MCHC	↓	↑	--	--	--
RBC Shape					
Discocytes	--	--	NC	NC	NC
Echinocytes	--	--	NC	NC	NC
Retics	↑	↓	↓*	↑	↓
BM Ery.	↓		NC	NC	NC
Fe Inc.	--	--	↓*	NC	NC
RBC Surv.	--	--	↓*	--	--
Epo	↓	--	↑	--	--

NC = not changed from control.

-- = not performed.

* = Sig. < 0.05

U.S.S.R. studies have also shown a decrease in hemoglobin mass of cosmanauts as shown in Figure 1.



Redrawn from Balakovskii et al. (1).

X axis, duration of (days).

Y axis, hemoglobin mass (% of base value).

The loss appears to level off so that after flights of 140 and 175 days mean decreases in hemoglobin mass (CO method) of -16 and -18 percent were found. Simulations by means of bedrest or immersion hypokinesia have shown a loss of red cell mass and plasma volume (10,13,14,17,20,24). The results of one study are shown in Table 7.

Table 7. Studies involving 30-day hypokinesia.

	HH ¹		AH ³		Control	
	Hgb M ⁴ g/m ²	Retic ⁵ %	Hgb M g/m ²	Retic %	Hgb M g/m ²	Retic %
Before	432	5.7	420	6.9	418	7.8
After 30 days	343 ⁶		350 ⁶		---	
14-17 d read pt.	---	13.0 ⁷	404	12.6 ⁷	420	9.4

From Balakovskii et al. (1).

¹ = horizontal hypokinesia

³ = antiorthostatic hypokinesia

⁴ = hemoglobin mass

⁵ = reticulocyte count

⁶ = $p < 0.02$

⁷ = $p < 0.001$

It is of interest that Kakurin et al. (7) found that antiorthostatic hypokinesia at -12° reproduced more closely the physiological responses shown in space crew members than did horizontal bed rest alone or head-down tilt at other angles.

In flight animals flown in space on Cosmos flights, Gazenko and Ilyin et al. found no differences in the values of hemoglobin, hematocrit and red blood cell counts (4,5). Gazenko et al. (4) and Leon et al. (16) found evidence for a hemolytic component in flight animals. However, in later studies, Leon et al. (15) found that when the animals were centrifuged in flight to produce the effect of gravity, the hemolysis was prevented indicating that the hemolysis results from the lack of gravity rather than other factors in the flight environment. Similar to the results of SL-3 rats, LeBlanc (unpublished observations) found that in flight animals there was an early post-flight significant increase in red blood cell counts, hemoglobin, hematocrit and mean corpuscular volume. Some observers have found a decrease in bone marrow red blood cell precursors in flight animals (4,5,21,23) though no changes were found in the SL-3 rats. It is apparent that findings for animal studies have varied from one study to the other and point up the need for isotopic studies of plasma volume and red cell mass to

determine if the changes mimic those observed in humans participating in microgravity flights.

We still do not know the cause of the reduction in red blood cell mass in astronauts 19 years after the first description of this phenomena by Fischer, Johnson and Berry (6) and whether the rat is a proper model for "space anemia." Lists of possible causes for the anemia have been published (6,26). The anemia may be caused and maintained by decreased production of red blood cells which could be multifactorial. Some of the accumulated data suggests that after an initial decrease in the circulating red cells, the "erythrostath" appears to be reset at a lower level due to a decreased demand brought on by weightlessness. This is analogous to the atrophy of disuse seen in muscles and other tissues and the body's setting of ideal points for organ weights. The need to perform isotope studies of red cell mass and plasma volume to determine if these values decrease as do those of human astronauts is inferred from the animal studies. The needs for future studies were pointed out by a working group of the life sciences research office of the Federation of American Societies of Experimental Biology and are given in Table 8 (25).

Table 8. Baseline data for analysis of erythrokinetics of space flights*

Parameter	Settings and Subjects			
	Ground-Based**		Inflight***	
	Animal	Human	Animal	Human
Red cell count	+	+	+	+
Hemoglobin	+	+	+	+
Hematocrit	+	+	+	+
Red cell mass	+	+	+	+
Blood volume	+	+	+	+
Plasma volume	+	+	+	+
Reticulocyte count	+	+	+	+
Erythropoietin	+	+	+	+
Plasma or serum				
haptoglobin	+	+	+	+
Platelets	+	+	+	+
Red cell shape	+	+	+	+
Red cell size	+	+	+	+
Blood P ₅₀	+	+	+	+
Blood PCO ₂	+	+	+	+
Red cell ATP	+	+	+	+
Red cell 2,3-DPG	+	+	+	+
Red cell sodium	+	+	+	+
Skin petechiae	-	-	+	+
Subcutaneous, subserosal				
oozing of RBC	-	-	+	-
Bone marrow smear	+	-	+	-

*When feasible, measure sequentially for temporal aspects.

**Examples: Biological laboratories, hospitals, space simulation facilities (bed rest, water immersion, etc.), spacecraft simulators.

***Include pre-, in-, and postflight phases.

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Effects of Weightlessness on Human Fluid and Electrolyte Physiology

CAROLYN S. LEACH AND PHILIP C. JOHNSON, JR.

Introduction

The fluid-regulating systems of the body have been of interest to space medicine researchers since results from the earliest flights indicated significant changes in this area (Berry et al. 1966; Dietlein and Harris 1966; Lutwak et al. 1969). The virtual absence of gravity causes a decrease in posturally induced hydrostatic force in the extremities, which leads to cephalad redistribution of blood. This redistribution is thought to be responsible for most of the spaceflight-induced changes in fluid and electrolyte metabolism. Plasma volume decreases (Johnson 1979) and water and electrolyte balances become negative (Leach 1979) in space travelers. In addition to these clear-cut effects, more complex and subtle changes in renal and circulatory dynamics, endocrine function, body biochemistry, and metabolism occur during spaceflight.

Two Phases of the Adaptation Process

Studies in which weightlessness is simulated by decreasing lower-extremity hydrostatic forces (as by bed rest or water immersion) have indicated the presence of at least two phases in the adaptation of the fluid and electrolyte homeostatic systems to microgravity (Leach et al. 1983). The "acute" phase is believed to occur within a few hours of attaining weightlessness. Since it has been difficult for astronauts to perform experiments early in a flight, most of the evidence for existence of this phase has come from simulation studies. Bed-rest studies (Leach et al. 1983; Nixon et al. 1979) have shown that central venous pressure (CVP) increases as early as 5 min after bed rest begins (Nixon et al. 1979). This is followed by an increase in the size of the left ventricle, but there is no change in cardiac output or arterial pressures (Nixon et al. 1979). The increased CVP is thought to be interpreted physiologically as an increase in total blood volume. Glomerular filtration rate (GFR) decreases by about 2 hr and effective renal plasma flow (ERPF) by 4 hr, but both return to pre-bed-rest levels by 8 hr. Plasma aldosterone and antidiuretic

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hormone (ADH) decrease between 1 and 6 hr after the beginning of bed rest (Leach et al. 1983; Nixon et al. 1979).

The transient acute phase, found in simulation studies and confirmed by recent Spacelab data to be discussed below, leads to a later "adaptive" phase. Evidence for the existence of the adaptive phase has come from blood and urine samples taken in-flight during Gemini, Apollo, Skylab, and Spacelab missions.

Early Spaceflight Findings

Data from limited in-flight samples, along with preflight and postflight measurements of many physiological parameters, provided evidence that mass is lost, water balance becomes negative, electrolytes and certain minerals are depleted, and cardiovascular deconditioning occurs as a result of weightlessness (Berry et al. 1966; Hoffler 1977; Leach et al. 1975). Fluid, potassium, and nitrogenous compounds were apparently lost from cells as well as from blood (Leach et al. 1975). Levels of some of the hormones involved in regulating fluid and electrolyte balance were altered; for example, urinary ADH and aldosterone and plasma angiotensin were increased postflight. Plasma volume and red cell mass decreased, and orthostatic tolerance and exercise capacity were reduced (Hoffler and Johnson 1975).

Skylab

Experiments for Skylab were planned to document the time course of known physiological changes and to measure additional parameters during long flights. Intake of fluid and nutrients during flight was carefully monitored.

The first in-flight measurements of body mass were performed on Skylab (Thornton and Ord 1977). The crew members lost an average of 2.8 kg, 3.8% of preflight body mass, during flight (Leach and Rambaut 1977). About half the loss of body mass occurred during the first 2 days of flight, with the rest of the loss being more gradual but continuing throughout the missions. Depletion of water was thought to be responsible for the rapid phase of mass loss and depletion of fat and protein for the slow phase (Leach and Rambaut 1977).

Increased urinary excretion of water was expected to account for the water deficit. Surprisingly, urinary excretion decreased during the first 10 days of the missions, and free water clearance decreased slightly (Leach and Rambaut 1977). Water balance studies showed that the main cause of the net body water reduction during the first 2 days of flight was a decrease in fluid intake.

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To investigate the postulated shift of fluid away from the lower extremities, the leg volume of Skylab crew members was measured by plethysmography (Thornton et al. 1977). It was estimated that in the first few days of flight, 1.8 l of fluid disappeared from the legs (Hoffler 1977), an amount considerably greater than the 600 to 800 ml redistributed by a change in body position (upright to supine or vice versa) (Sjöstrand 1953). The amount of fluid lost from the legs was almost equal to the total body fluid decrement.

The loss of so much body fluid implies that levels of other blood and tissue components are reduced also. Plasma osmolality and levels of sodium and chloride were decreased during flight, and the amounts of sodium, potassium, calcium, phosphate, and magnesium were increased in 24-hr urine pools collected in-flight. Leach and Rambaut (1977) calculated that approximately 100 meq of sodium were lost from the extracellular space. Electrolytes and other cell constituents may have been translocated from cells to blood. Plasma levels of potassium, calcium, and phosphate increased during flight.

Plasma angiotensin and urinary aldosterone and cortisol were increased over their preflight levels during the whole flight but were particularly increased at the beginning of each flight (Leach and Rambaut 1977). These hormones are released in response to stress and to changes in plasma osmolality and electrolytes. Increased angiotensin and aldosterone may have caused at least part of the increased urinary excretion of potassium, but it is unusual for high levels of aldosterone to be associated with increased sodium excretion. Urinary excretion of antidiuretic hormone (ADH) was decreased during flight, another unexpected finding because the loss of fluid would normally stimulate ADH secretion, and hyponatremia persisted in spite of the apparent reduction in ADH.

Renal function was not measured directly during the Skylab flights. Slight increases in creatinine clearance (Leach 1981), decreased urinary and plasma uric acid, and increased plasma angiotensin indicated that renal function may be affected by weightlessness.

Recent Findings from the Space Shuttle

Several experiments involving fluid and electrolyte physiology have now been performed aboard the space shuttle. Venous pressure was measured for the first time on *Spacelab 1*, 22 hr after launch (Kirsch et al. 1984). At that time venous pressure was lower than it was on the day before launch. Measurement of central and peripheral venous pressures 1 and 12 hr after landing indicated that fluid redistribution after reexposure to gravity was completed between these times. If redistribution caused by microgravity takes about the same amount of time, it is probably complete before 22 hr.

Studies of body fluid changes during spaceflight have been hampered by lack of knowledge about changes in circadian rhythm and by flight-related

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problems such as space adaptation syndrome or crew members being on different work/rest cycles or being unable to draw blood very soon after reaching orbit or at the same time each day.

In one experiment a mission specialist collected his urine as pools representing 5 to 26 hr, and the excretion rates of electrolytes and selected hormones were determined. The earliest change detected in this study was a transient increase in the excretion rate of ADH in the first in-flight sample. This was closely followed by a transient increase in the excretion rate of cortisol. Sodium excretion decreased on the day after the peak in cortisol excretion occurred, but later in the flight it increased. Potassium excretion increased at the same time as cortisol excretion on the first day of flight, with smaller peaks on later flight days. Some of these changes may have been caused or affected by the presence of space adaptation syndrome. On the sixth and last day of the flight, aldosterone excretion rate tripled, and cortisol and ADH excretion rates increased by lesser amounts. The excretion rates of fluid, potassium, chloride, calcium, and magnesium increased at the same time. The loss of sodium, which might be expected to result in increased aldosterone secretion, was no greater late in the flight than it had been during the preflight period.

On the Spacelab flights, blood samples were drawn 22 or more hours after launch. Aldosterone, cortisol, and ADH were measured in blood samples from Spacelab 1 by Dr. K.A. Kirsch (personal communication), and our laboratory has measured these and other hormones as well as serum osmolality, sodium, and potassium in other Spacelab experiments. The four crew members on Spacelab 1 who participated in experiments were on two different work/rest cycles, but their blood samples were taken at the same clock time, and studies of the circadian rhythms of several urinary variables showed that the circadian rhythm of metabolic functions did not change (Leach, Johnson, & Cintrón 1985). Blood samples were obtained from four mission and payload specialists on Spacelab 2 and two mission specialists on Spacelab 3. The crew members on Spacelab 2 were on two different work/rest cycles, and during flight they collected blood samples during the postsleep activity period. This was 6:30 or 7:00 a.m. Houston time for two crew members and 5:00 or 6:00 p.m. for the other two. Because of differences in sample collection times, one must be cautious in interpreting the results, but the small number of subjects and time points in any one experiment makes it desirable to examine the results of these three experiments together.

The combined results for all three Spacelab studies (Leach et al. 1985) showed that hyponatremia developed within 20 hr after the onset of weightlessness and continued throughout the flights, and hypokalemia developed by 40 hr. Serum potassium returned to preflight levels later and then increased. Serum chloride was decreased on most in-flight days on which it was measured, but it immediately returned to preflight levels on landing day.

Antidiuretic hormone, which increased transiently in urine in the

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shuttle experiment described above and decreased in urine during Skylab flights (Leach and Rambaut 1977) and in plasma during bed rest (Nixon et al. 1979), was increased in plasma throughout the Spacelab flights. Aldosterone decreased by 40 hr, but after 7 days it had reached preflight levels. Angiotensin I was elevated after 2 days in flight. Cortisol increased early but decreased later in the flight. Adrenocorticotrophic hormone was increased until the seventh day, when levels of cortisol and aldosterone returned to or surpassed baseline.

Current Problems

The changes that occur in human fluid and electrolyte physiology during the acute and adaptive phases of adaptation to spaceflight are summarized in Tables 11.1 and 11.2. A number of questions remain to be answered.

At a time when plasma volume and extracellular fluid volume are contracted and salt and water intake is unrestricted, ADH does not correct the volume deficit and serum sodium decreases. Change in secretion or activity of a natriuretic factor during spaceflight is one possible explanation.

Recent identification of a polypeptide hormone produced in cardiac muscle cells which is natriuretic, is hypotensive, and has an inhibitory effect on renin and aldosterone secretion (Atarashi et al. 1984; Palluk et al. 1985) has renewed interest in the role of a natriuretic factor. The role of this atrial natriuretic factor (ANF) in both long- and short-term variation in extracellular volumes and in the inability of the kidney to bring about an escape from the sodium-retaining state accompanying chronic cardiac dysfunction makes it reasonable to look for a role of ANF in the regulation of sodium during exposure to microgravity. Prostaglandin E is another hormone that may antagonize the action of ADH (Anderson et al. 1976). Assays of these hormones will be performed on samples from crew members in the future.

TABLE 11.1. Acute phase of actual or simulated microgravity effects on fluid and electrolyte physiology

Cardiovascular effects (bed rest)
Increased central venous pressure
Increased size of left ventricle
Renal effects (bed rest)
Decreased GFR
Decreased ERPF
Endocrine system changes (Spacelab)
Increased plasma cortisol
Decreased plasma angiotensin I

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TABLE 11.2. Adaptive phase of microgravity effects on fluid and electrolyte physiology, compared with preflight.

Mass loss
Water
Protein
Fat
Changes in fluid volumes in major body compartments
Decrease in lower body
Increase in upper body
Decrease in intracellular water
Decrease in extracellular fluid
Negative fluid balance
Decreased fluid intake
Increased evaporative water loss
Decreased renal excretion of water
Slightly decreased free water clearance
Decreased total body water
Electrolyte balance
Decreased exchangeable body potassium
Decreased sodium in extracellular space
Increased excretion of sodium
Increased excretion of potassium
Blood levels of electrolytes
Increased potassium
Decreased sodium
Decreased chloride
Decreased osmolality
Endocrine system changes
Increased plasma angiotensin I
Increased urinary aldosterone
Increased urinary cortisol
Increased plasma ADH
Decreased urinary ADH
Renal function
Decreased plasma and urinary uric acid
Increased creatinine clearance

Cardiovascular intolerance to standing, found to occur immediately after landing in many astronauts, is thought to be related to loss of fluid and electrolytes during weightlessness. In the space shuttle, reentry acceleration is experienced in the head-to-foot direction because the crew members are sitting upright. The gravitational force in that direction is usually about 1.2 times the normal 1.0 *G* (Nicogossian and Parker 1982). The rapid increase in acceleration forces during reentry would be expected to pull body fluids toward the legs. If there has been substantial loss of body fluid, fluid volume in the upper part of the body may decrease enough to cause cardiovascular symptoms. Some of these symptoms might be alleviated if fluid and electrolyte metabolism were fully re-

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adapted to earth's gravity before landing. Attempts have been made, with some success, by investigators in the United States and Soviet space programs to prevent or ameliorate orthostatic intolerance.

One method that has been used to counter the orthostatic intolerance is fluid and electrolyte loading. If fluid and electrolytes are replaced, blood volume should begin to increase and blood pressure should approach preflight levels. This should be done before exposure to the increased acceleration during the deorbit period. It is now standard practice for U.S. astronauts to consume the equivalent of a liter of physiological saline solution in the form of water and salt tablets before landing is initiated. This practice has been shown to be effective in reducing the severity of symptoms of cardiovascular deconditioning (Bungo et al. 1985). Similar countermeasures have been used by cosmonauts on Soyuz missions (Grigoriev 1983).

Another approach to prevention of postflight orthostatic intolerance is the use of lower-body negative pressure (LBNP) during flight to bring more fluid into the legs; this has been used with some success in the Soviet space program (Grigoriev 1983).

There is now considerable indirect evidence that renal function is altered during weightlessness (Leach, Johnson, & Cintrón 1985), but direct measurements of renal function have been done only in bed-rest studies. Renal function tests will be performed in conjunction with measurement of hormones, electrolytes, plasma volume, and other factors on Spacelab missions in the future. Intake of food and water will be measured throughout the mission, and urine will be collected void by void. Blood samples will be taken at intervals, beginning at 3 hr after launch, and the first renal function test will start at 3.5 hr. A catheter to measure central venous pressure will be inserted before launch and removed 12 hr into the flight. Plasma volume and extracellular fluid will be measured on the second and sixth days of flight. These integrated experiments are expected to provide information important for understanding what happens in both phases of the fluid and electrolyte response to weightlessness.

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THE ENDOCRINE SYSTEM IN SPACE FLIGHT†

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Abstract—Hormones are important effectors of the body's response to microgravity in the areas of fluid and electrolyte metabolism, erythropoiesis, and calcium metabolism. For many years antidiuretic hormone, cortisol and aldosterone have been considered the hormones most important for regulation of body fluid volume and blood levels of electrolytes, but they cannot account totally for losses of fluid and electrolytes during space flight. We have now measured atrial natriuretic factor (ANF), a hormone recently shown to regulate sodium and water excretion, in blood specimens obtained during flight. After 30 or 42 h of weightlessness, mean ANF was elevated. After 175 or 180 h, ANF had decreased by 59%, and it changed little between that time and soon after landing. There is probably an increase in ANF early inflight associated with the fluid shift, followed by a compensatory decrease in blood volume. Increased renal blood flow may cause the later ANF decrease. Erythropoietin (Ep), a hormone involved in the control of red blood cell production, was measured in blood samples taken during the first Spacelab mission and was significantly decreased on the second day of flight, suggesting also an increase in renal blood flow. Spacelab-2 investigators report that the active vitamin D metabolite $1\alpha, 25$ -dihydroxyvitamin D_3 increased early in the flight, indicating that a stimulus for increased bone resorption occurs by 30 h after launch.

1. INTRODUCTION

Recent evidence suggests that the physiologic responses to space flight begin immediately. On the basis of weightlessness simulation studies[31] and findings that leg volume decreases and the face becomes puffy inflight[36], it has been proposed that the removal of the gravitational force on the legs results in an early and transient increase in central blood volume. On the Space Shuttle such a fluid redistribution may begin even before weightlessness is achieved. The launch configuration causes Space Shuttle crewmembers to recline for several hours before launch, and during ascent to orbit, the Shuttle acceleration angle produces $-g_z$ forces on the crew in their seats. Evidence from the D-1 Spacelab mission on the Space Shuttle indicates that fluid redistribution does begin before launch: central venous pressure, when measured beginning 20 min after launch, never exceeded preflight measurements[18]. By the end of the first 24 h of flight, changes have occurred in many physiological systems, especially those related to the distribution of fluid in the body. In most systems, these changes are relatively rapid and a new steady state seems to have been reached.

Because of the importance of the endocrine system in regulation of the body's homeostatic mechanisms, a number of hormones have been studied in the blood and urine of crewmembers during and after space flight. Hormones involved in maintenance of body fluid compartments, erythropoiesis, mineral phys-

iology, and regulation of metabolism have had primary focus in the U.S. manned space flight program.

2. FLUID AND ELECTROLYTE REGULATION

Early studies showing loss of weight[14] and a decrease in blood volume and interstitial volume of the lower extremities[15] after space flight indicated that a loss of body fluid occurred during flight. The translocation of fluid from the extremities to the head and chest during space flight is thought to cause a transient increase in central blood volume and central venous pressure. The results of Kirsch *et al.*[18] suggest this is complete prior to the first inflight measurement, while the echocardiographic data of Pourcelot *et al.*[32] and Bungo *et al.*[4] indicate it takes less than 6 h. The increase in central venous pressure is detected by stretch receptors in the heart and superior vena cava and interpreted as an increase in total blood volume. A compensatory loss of water and sodium results. Further investigation has revealed other effects of space flight on fluid physiology and some of the control mechanisms that mediate those effects.

Body composition of the Skylab astronauts was studied in detail before, during and after flight[28]. Water accounted for about half of the weight lost during flight. Although urinary excretion of fluid did not increase, fluid intake decreased to cause a negative water balance[26]. Urinary sodium, potassium, and chloride generally increase in microgravity, with a concomitant increase in urine osmolality, while serum osmolality and sodium are decreased throughout flight[23,26]. In Skylab crewmembers, plasma potassium was slightly increased during most of the

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time inflight, but in Spacelab crewmembers, serum potassium was reduced for several days between 30 and 120 h after launch. The maintenance of serum hypo-osmolality and hyponatremia along with increased sodium excretion is surprising because normally these conditions would be corrected by homeostatic regulatory mechanisms including hormones.

Several hormones are known to participate in regulation of body fluid volume and blood levels of electrolytes. For many years antidiuretic hormone (ADH), cortisol, and aldosterone have been considered the most important hormones performing these functions. However, measurements of these compounds in inflight specimens have indicated that their levels cannot account for the entire measured losses of fluid and electrolytes.

Recent Spacelab experiments have shown that ADH increased in plasma during flight[23], but urinary ADH decreased in most Skylab crewmembers relative to preflight levels[26], raising the possibility that the low serum osmolality might have another cause. A transient increase in urinary ADH on the Shuttle[20] may have resulted from symptoms of space motion sickness in the one crewmember whose urine was collected. Space motion sickness, which affects most of the crewmembers, is a confounding variable in the analysis of data resulting from samples obtained during the first 2 days of a flight.

The levels of aldosterone and angiotensin I in inflight samples were particularly inconsistent. Plasma aldosterone did not change significantly during the Skylab flights[26], but in Spacelab astronauts it decreased[23]. Urinary aldosterone, on the other hand, increased in both Skylab[26] and Shuttle astronauts, suggesting increased clearance of the hormone.

Angiotensin I, a hormone that stimulates aldosterone production, increased early during Skylab flights, then returned to preflight levels and continued to decrease for several weeks[26]. On Spacelab missions, plasma angiotensin I was reduced for 2 days and then increased[23].

Plasma and urinary cortisol were increased over preflight levels throughout the Skylab flights[26], but on the Space Shuttle both were elevated for a few days, then decreased to preflight levels or below[20,23]. Cortisol may be particularly important because it has been implicated as a cause of bone calcium loss in humans; this will be discussed further in another section. Adrenocorticotropin decreased at various times during Skylab flights[17], but on the Spacelabs it was increased for at least a week[23].

Additional variables must be measured before the picture of fluid regulation in space can be completed. Low serum potassium and the persistence of lower serum sodium during flight cannot be explained in terms of observed angiotensin I, ADH, cortisol, and aldosterone levels. The electrolyte data might be partially explained by increased activity of a natriuretic factor during flight or by an increase in renal blood flow. Renal blood flow has not yet been measured during flight. Since atrial natriuretic factor (ANF) has now been recognized as a hormone that regulates sodium and water excretion, we have measured ANF in blood specimens obtained during and after the Spacelab-2 mission.

Blood samples were collected from 4 astronauts on this mission on 3 days preflight, 2 days inflight, and 3 days postflight. The first time it was measured after launch, 30 or 42 h into the flight, ANF increased 36% over the preflight mean (Fig. 1). After 175 or 185 h of weightlessness, ANF had decreased by 59%, and

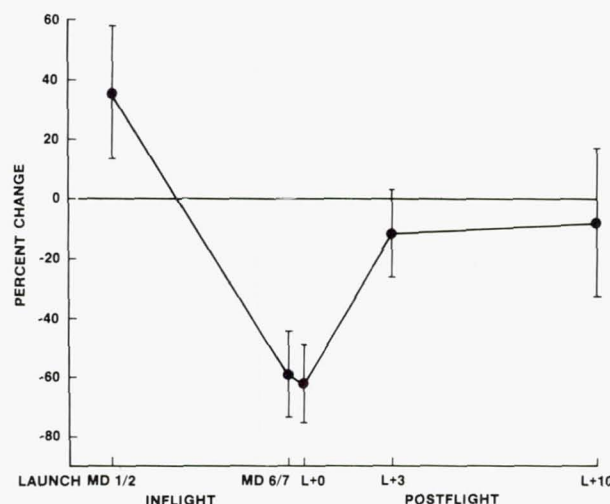


Fig. 1. Mean percent change in plasma ANF during and after the Spacelab 2 mission. Blood samples were collected in ethylenediaminetetraacetic acid (EDTA), and plasma and red cells were separated before freezing. Plasma samples for ANF assay were extracted with a Sep-Pak C18 cartridge[35]. Atrial natriuretic factor was determined by radioimmunoassay[12]. For each of 4 crewmembers, each measurement was compared to the mean for 3 preflight days. Bars represent standard error.

it changed little between that time and immediately (1.5 h) after landing. Three days after landing, plasma ANF had increased to preflight level in all crewmembers.

Although urine data are not available for this flight, a number of other variables were measured in blood samples. Sodium had decreased about 1% late in the flight, but was at preflight level immediately after landing. Potassium, on the other hand, increased 9% after 175-185 h of weightlessness, then decreased slightly after landing. Plasma osmolality did not follow the pattern of either of these electrolytes: it decreased during flight, particularly on the earlier day (4%). Osmolality returned to preflight level at landing. The ingestion of a liter of water and eight salt tablets several hours before landing, used as a countermeasure against orthostatic intolerance, occurred after the later inflight blood collection but probably influenced levels of electrolytes and hormones in samples taken on landing day.

Several other hormones were measured in plasma samples from Spacelab 2 crewmembers. Angiotensin I decreased by 40% at the early inflight sampling time, but by the later sampling time it had increased by 40%. It increased even more at landing, then decreased during the recovery period. Aldosterone had a similar pattern of change, but it decreased only about 10% early in the flight and was at preflight level late in the flight while angiotensin I was elevated. Cortisol was increased over preflight levels at all times during and after the flight, especially on the second mission day and immediately after landing. Even 10 days after landing, cortisol was 50% higher than it had been before flight, and the increase was highly consistent in all four crewmembers. This pattern was more similar to that of Skylab crewmembers than to cortisol measurements from other Spacelab missions[23,26]. Adrenocorticotropin, the pituitary hormone that stimulates secretion of cortisol, increased similarly but generally not as much as cortisol.

An increase in plasma ANF has been observed in weightlessness simulation experiments[10], with a maximum at 30 minutes after the beginning of head-down bedrest. These results indicate that 30 h after launch is too late to measure the highest levels of plasma ANF during actual weightlessness. As discussed above, fluid redistribution in Shuttle crewmembers may occur before launch. Release of ANF in humans can be caused by increased blood volume[38] a condition that would be sensed for a short time when fluid shifts to the head and chest. An increase in atrial pressure, which would result from increased blood volume, is thought to stimulate ANF release[13]. Measurement of the left atrium of astronauts by echocardiography indicates that atrial size increases very early during flight but decreases later and returns to normal after landing (Dr. John Charles, personal communication). Atrial pressure has not yet been measured during weightless, but increased atrial

dimensions indicate that atrial pressure may be increased. Another possible cause of ANF increase is suggested by the finding that ADH, which was found at increased levels in blood plasma of astronauts on Spacelabs 1 and 3, can stimulate release of ANF in the rat [29]

After 6 or 7 days of weightlessness, adaptation of most physiological systems has occurred; red cell mass, plasma volume [22], and atrial pressure are probably reduced.

Although crewmembers ingest a liter of physiological saline before landing and the return to Earth's gravity might be expected to affect cardiovascular variables, echocardiographic studies show that atrial dimensions are decreased just after landing (Dr John Charles, personal communication), so that decreased ANF might be expected. Blood volume does not fully recover for at least 2 weeks, and restoration of preflight vascular tone may not occur immediately because distension of the veins may result from low venous pressure.

Atrial natriuretic factor promotes excretion of sodium and water [33] and inhibits secretion of renin[5] and aldosterone[2]. Early release of excessive amounts of ANF may explain natriuresis early in flight, but the continued loss of sodium apparently cannot be explained by ANF, since circulating ANF decreases later. Higher levels of ANF would probably exacerbate the hyponatremia that begins after a day or more of flight. It is possible that the decreased

ANF contributes to an increased level of angiotensin I at 175-185 h. Although the aldosterone level at that time does not reflect the increase in angiotensin, it is possible that aldosterone production is increased or unchanged and it is being excreted at an elevated rate as observed in Skylab astronauts.

The action of still other hormones may help to explain changes in fluid and electrolyte metabolism during space flight. Prostaglandin E antagonizes the action of ADH[1] and will be measured during space flight and bedrest. There is evidence that circulating prostaglandin levels are influenced by body position: prostaglandin #2 increased in renal venous blood when subjects sat up after being supine[16]. Prostaglandins may also be involved in bone mineral metabolism.

The changes in fluid and electrolyte metabolism in space flight indicate that weightlessness may affect kidney function. Because of the invasiveness of standard techniques for measuring such variables as renal blood flow and glomerular filtration rate (GFR), these variables have not yet been measured in space. However, our laboratory has measured GFR and effective renal plasma flow (ERPF) during head-down bedrest, and ERPF had decreased 26% after 4 h[25]. Eight hours after bedrest began, both variables had increased to pre-bedrest levels or higher. Creatinine clearance studies performed during the Skylab flights indicated that creatinine clearance and therefore GFR increases slightly[19].

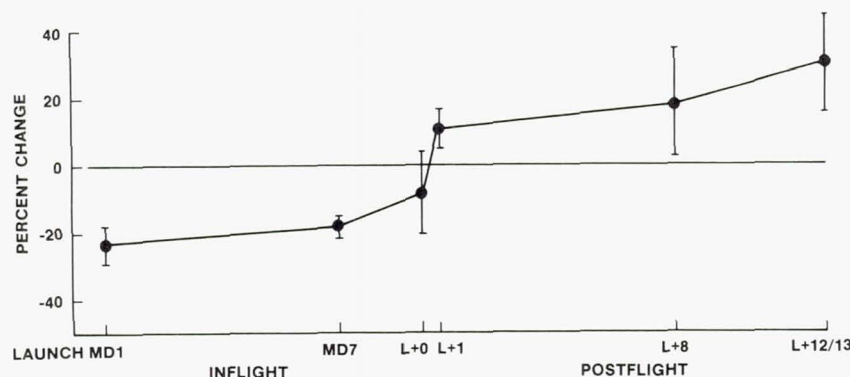


Fig. 2. Mean percent change in plasma erythropoietin during and after the Spacelab 1 mission. For each of 4 crewmembers, each measurement was compared to the mean for 3 flight days. Bars represent standard error.

If increased renal blood flow were characteristic of physiological adaptation to weightlessness, it might explain the continued increased excretion of electrolytes and the hyponatremia. Spacelab missions dedicated to the life sciences should allow the necessary renal experiments to be performed in space. On the first and second Spacelab Life Sciences missions, renal blood flow, glomerular filtration rate, central venous pressure, plasma volume, extracellular fluid, and a number of hormones and electrolytes in blood and urine will be measured as part of the same experiment. Food and water intake will be recorded during the mission, and all urine voids will be collected. Beginning at 3 h after launch, blood will be drawn at carefully controlled intervals. The first renal function test will start 3.5 h after launch, and central venous pressure will be measured from before launch to 12 h after launch. On the second and sixth days of flight, extracellular fluid and plasma volume will be measured. The results of this experiment should indicate how renal blood flow is affected by weightlessness and how it might affect other physiological variables.

3. ERYTHROPOIESIS

A decrease in red cell mass is one of the most consistent findings in astronauts immediately after flight, and at least 2 weeks are required for recovery. An experiment was done on Spacelab-1[22] to determine whether the reduction in red cell mass is caused by decreased production or increased destruction of red blood cells. Erythropoietin (Ep), a hormone that stimulates red blood cell production, was measured as part of this experiment, the first time this hormone has been measured in blood samples taken during space flight.

Preliminary results were obtained by use of the fetal mouse liver assay, but this method was not considered to be sensitive enough to measure suppressed physiological levels of the hormone. When a radioimmunoassay method[7] became available, it

was used to determine Ep level in three preflight, two inflight, and four postflight frozen samples from each of four crewmembers who participated in the study. When statistical contrasts were performed on pairs of consecutive days, it was found that the first inflight measurement (the second day of flight) was significantly lower (Fig. 2) than the last preflight measurement (1 day before launch). Blood levels of Ep were still reduced a week after launch and immediately after landing. The crewmembers had an average 15% decrease in red cell mass immediately after landing. There was a significant positive correlation between erythropoietin and reticulocyte number in this experiment, indicating that production of red blood cells was associated with erythropoietin level.

Production of Ep is affected by renal plasma flow, which has not yet been measured during space flight but may be increased most of the time during weightlessness. Reduction of renal blood flow by renal artery constriction has been shown to increase Ep production[8,9], but the reverse experiment has not been published because of the difficulty of measuring low levels of Ep in plasma. The relationship of plasma erythropoietin and renal blood flow in microgravity will be investigated when both are measured on the same integrated Spacelab Life Sciences mission.

4. CALCIUM METABOLISM

Parathyroid hormone and 1,25-dihydroxyvitamin D₃ have been the main focus of recent NASA studies of mineral physiology in flight. Circulating levels of these hormones measured during space flight and ground simulation have varied with duration of flight. However, in all cases calcium has been lost from the body.

Preliminary results of an experiment performed on Spacelab-2[34] indicate that levels of two metabolites of vitamin D, 25-hydroxyvitamin D₃ and 24,25-dihydroxyvitamin D₃, did not change significantly during flight. The metabolite 1 α ,25-dihydroxyvitamin

D₃, which is produced by the kidney and increases intestinal calcium absorption and resorption of calcium from bone, increased substantially in the first 2 days of flight. This suggests that the 1 α ,25-dihydroxyvitamin D₃ may help initiate the calcium loss from bone. In Spacelab-2 crewmembers who participated in the experiment, plasma calcium was indeed elevated early in flight when 1 α ,25-dihydroxyvitamin D₃ increased, and it returned to preflight levels late in the flight and at landing. During the postflight recovery period, however, when 1 α ,25-dihydroxyvitamin D₃ was only slightly decreased, plasma calcium exhibited its greatest change, a 5% decrease, which was probably associated with changes in other plasma electrolytes measured after the flight.

Investigation of the role of key metabolic hormones such as insulin, thyroxine and catecholamines is necessary for thorough study of calcium metabolism. The changes measured in insulin during space flight have indicated that a net catabolic state may exist[21]. Thyroxine has not yet been measured in blood samples drawn in flight. Comparison of preflight and postflight plasma thyroxine shows that thyroxine increased 11.5% and that this increase was highly significant ($P < 0.00001$). Triiodothyroxine decreased slightly, and thyroid stimulating hormone increased significantly (21.5%, $P < 0.005$). These results agree with those of Leach, Johnson, and Driscoll[24] for Skylab. Increased thyroxine during flight would probably contribute to a catabolic state and promote muscle atrophy and bone resorption. Negative calcium balance, hypercalcemia, and bone diseases such as osteoporosis have been observed in hyperthyroid patients[39], and thyroid hormone stimulates bone resorption *in vitro*[30].

Although high plasma levels of cortisol have been associated with bone mineral loss in humans under certain conditions, recent evidence from experiments in which weightlessness is simulated indicate that increased blood levels of glucocorticoids are not the main cause of bone mineral loss during space flight. In a suspended rat model in which only the hindlimbs were unweighted, calcium was lost only from the unweighted limbs[11]; if glucocorticoid had been a major cause of calcium loss, the loss probably would have been ubiquitous. Adrenalectomy did not prevent the decrease in bone mass in this model[3]. In human studies using bedrest as a weightlessness simulation, there is usually no increase in plasma cortisol[27]. Plasma cortisol did not change in cosmonauts on the Salyut-6 mission, but a loss of bone density was measured[37].

Calcium excretion may be influenced by many factors that affect sodium excretion. When diuresis is induced by loading with water or sodium chloride, and during acetylcholine-induced vasodilation, clearances of both sodium and calcium increase[6]. Calcium and sodium are excreted similarly when renal hemodynamics are altered. It is possible to dissociate

renal excretion of these two ions by such actions as infusing parathyroid hormone, which increases calcium but not sodium excretion, and administering mineralocorticoids, which decrease sodium but not calcium excretion. Urine samples were not available from Spacelab-2, but plasma sodium and calcium did not change in the same direction, and in Skylab crewmembers urinary sodium and calcium did not change proportionately[26].

5. CONCLUSION

Our studies to date demonstrate the complexity of the systems that control fluid and electrolyte metabolism, erythropoiesis, and calcium metabolism. These areas are some of the most important in space medicine because of weightlessness effects such as orthostatic intolerance and decreased bone density. Future experiments on Spacelab missions and the Space Station will test the current working hypotheses about causes and prevention of problems associated with return to the 1-g environment.

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MATH MODELLING AS A COMPLEMENT TO THE SCIENTIFIC INQUIRY OF PHYSIOLOGICAL ADAPTATION TO SPACE FLIGHT: FLUID, ENDOCRINE AND CIRCULATORY REGULATION

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ABSTRACT

This paper discusses the contribution that mathematical modeling and computer simulation have made to the understanding of some of the problems arising during weightless space flight. A number of examples are provided from the areas of fluid, endocrine and circulatory regulation to illustrate the utility of modeling as an adjunct to the process of scientific inquiry, especially in the development and theoretical testing of hypotheses. The models were used to examine both the acute and chronic phases of space flight. Many paradoxical results appear when data obtained during chronic adaptation is interpreted using theories which pertain to acute adjustments. The modeling process has provided a means of developing a theoretical basis for interpreting the chronic adaptive phase of flight.

Keywords: mathematical models, computer simulation, physiological adaptation to microgravity, fluid regulation.

1. INTRODUCTION

Mathematical modeling is generally useful for studying systems that are poorly understood, complex and data poor. It is also valuable in situations in which experimentation is costly or impossible. This would certainly seem to apply for the field of space physiology which is still in its infancy. For these reasons, mathematical modeling and computer simulation have been in use by NASA for a number of years as one approach for understanding physiological adaptation to microgravity (Ref. 1). Modeling has been used principally as a means of organizing and interpreting the data within a given discipline. But also it has proved useful in integrating data across disciplinary lines because adaptation to microgravity certainly is the result of changes from a large number of physiological systems acting in concert. We believe modeling has contributed to understanding these changes and this paper will describe a portion of this effort.

Although a number of examples and approaches will be discussed, the underlying theme is that modeling is a true complement to the traditional process of scientific inquiry, in a manner similar to the use

of experimental models. Models, experimental or mathematical, are certainly capable of suggesting explanations and making predictions regarding the system under study. However, those explanations and predictions are often not as important as the questions we are forced to ask in order to understand why a model behaves as it does or why it doesn't behave as we think it should. Thus models should be judged, not solely on the basis of their ability to predict future events but on the power they offer in raising the critical questions. These critical questions then become the basis for other key aspects of the scientific process-hypotheses development and the experiments to test them.

The task of developing a sound understanding of the space-flight adaptation processes is confounded by the lack of adequate data in some cases and by an incomplete physiological epistemology in other cases. Modeling however, provides a powerful framework in which to place what is known about a particular situation (data), or about a physiological system (relationships), along with what is suspected about that system (hypotheses) and permits us to assess their consistency under many situations.

The examples of modeling which are presented here are all concerned with fluid-electrolyte metabolism and the renal-endocrine control of that system as it adapts to a new environment. Two particular mathematical models have been especially useful in this effort and these will be summarized briefly in the next section.

2. MODELS AND TECHNIQUES

A model which has proved invaluable in these studies was developed some time ago by Arthur Guyton for the study of overall circulatory, fluid, and electrolyte regulation (Ref. 2). This model contains subsystems which describe fluid and electrolyte exchanges between the major volume compartments, cardiovascular and renal dynamics, hormonal control, and blood volume regulation including separate control of plasma and red cell components. Both short-term and long-term adaptive control mechanisms are represented. Recent modifications include adding leg vascular and tissue compartments, gravity dependent circulatory elements, and a natriuretic factor. These additions provide the capability for simulating postural

changes and fluid shifts between upper and lower body. The entire biological control system represented in this model is extremely complex but it offers to the physiologist an opportunity to examine complex interactions, simultaneous stimuli, and steady state as well as dynamic behavior.

A second and less complex model was developed by Leonard et al (Ref. 3) to examine the more limited area of erythropoiesis control. The model incorporates the current understanding of the dynamics of red cell production and associated feedback regulation based on the balance between tissue oxygen supply and demand. Simulation studies with this model have provided useful insights into the still perplexing problem of the "anemia" of space flight. It is possible to use this model on a stand-alone basis, and it has also been incorporated into the modified Guyton model. The Guyton and Leonard models together form the theoretical basis to study total blood volume regulation in space flight (Ref. 4).

The simulation of headward fluid shifts in the modified Guyton model is most easily simulated by altering the angle of tilt. This changes the direction and magnitude of the gravity vector as it affects blood pooling in the legs. Once the angle of tilt is specified and the model simulation begins, fluids become redistributed by the elastic forces of the tissues and vessels in a manner favoring upper body hypervolemia. Subsequently, feedback controllers respond by regulating blood flow, blood pressure, and blood volume toward more normal values.

Simulations of space-flight using the stand-alone model of erythropoiesis were accomplished by simply removing about 10 percent of the plasma volume. (The plasma volume in this model is fixed and controlled by the user, while it is adjusted homeostatically in the Guyton model). The resulting hemoconcentration provides an appropriate hyperoxic disturbance that results in a realistic simulation of the flight data.

The practical approach for using these models is illustrated in Figure 1. Data from space flight or ground-based analog studies are used for two purposes. First, to generate hypothesis and second, to verify the models by comparing the theoretical and actual responses. If required, the model's responses could be altered, by formulating one or more hypotheses, and modifying the model in accord with this formulation. Computer simulation techniques could then be applied to test these hypotheses and assess their plausibility. These techniques might include merely changing the value of a fixed parameter (i.e., adjusting the gain or set point of a control loop), clamping the value of a variable (i.e. opening a feedback loop), or in some cases, introducing an entirely new control mechanism into the model. The most plausible of these results may then become a starting point for the design of validation experiments in the laboratory and eventually lead to permanent improvements of the model. This interaction between model simulation and experiment studies is valuable and synergistic. The approach discussed above is known as hypothesis development and testing and is used principally to help interpret experimental data. Some of the benefits which have been derived from hypothesis development include the ability to explain paradoxical behavior, to assess alternate hypotheses, to predict difficult to measure quanti-

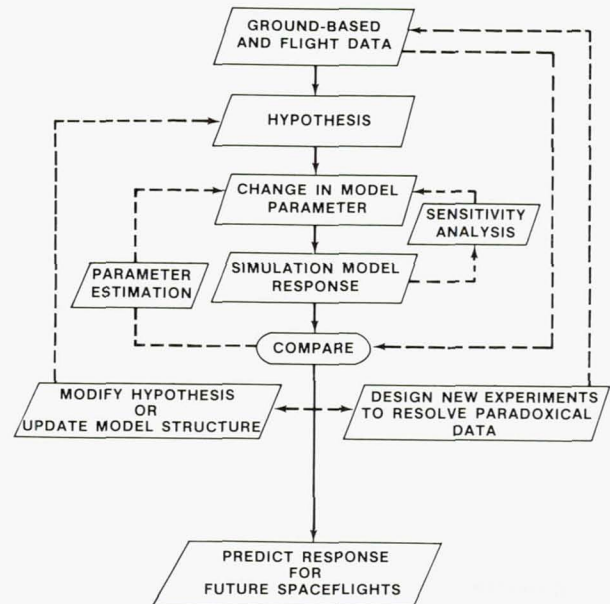


Figure 1. Use of simulation models in space flight program

ties, to isolate mechanisms and physiological pathways, and to synthesize scenarios consisting of multiple hypotheses.

Another approach to modeling, is often employed to study the feedback control behavior of the underlying physiological system. While the former approach requires rigorous model-to-data comparison, the study of system behavior often is characterized by model-to-model comparison and therefore does not require new data in addition to that used to formulate the model. In Figure 1, this system behavior approach to modeling is represented by the "sensitivity analysis" technique. Sensitivity analysis is used to reveal those parameters which have the most sensitive influence on a system. Other similar techniques that have been used include parametric analysis, dynamic vs steady-state analysis (to look for non-linear effects), and feedback stability analysis. Finally, a third approach is the "parameter estimation" technique in Figure 1 and is a means of determining the value of a model parameter which produces an optimum "fit" between model and experimental responses. Of all the techniques mentioned, this method of curve-fitting is most commonly employed by physiological simulation analysts. It has not been widely used in the space-flight studies, however, and is included here only for completeness.

The remainder of the paper will discuss the results of modeling analyses in addressing specific problems in the areas of acute fluid loss, blood volume regulation, control of central venous pressure, and hormone behavior.

3. ACUTE FLUID SHIFTS

Perhaps the most dramatically visible event that occurs to the physiological systems immediately upon entering microgravity is the shift of fluids, which are usually pooled in the legs by gravitational forces, to the upper body. Associated with the measured reduction in leg volume is the observation of distended cephalic veins and tissues. This shift of fluids is thought to lead to a sus-

Theoretically, it is possible for a reduction in plasma volume to occur by any one of the three major routes shown in Figure 2. In practice, it appears that each pathway's contribution depends on its unique characteristics and the circumstances of the experimental study. For example, on Skylab most of the fluid losses can be accounted for, mathematically at least by deficit drinking (Refs. 6, 9) while during water immersion and head-down tilt studies, fluid losses are related to a renal diuresis. In view of the ground-based results, the lack of a measured diuresis during the first inflight day has been particularly puzzling. However, computer modeling studies have suggested that when fluid intake is restricted (as exhibited during the first few days by most space crews), an acute diuresis can be obscured in a 24-hour pooled urine collection because of a subsequent anuresis. These theoretical studies also predicted that while restricted drinking contributes to a reduction in body fluids, it does not abolish the diuresis effect completely; accurate measurement of the diuresis may require careful and continuous monitoring. Conversely, it is predicted that there will be an easily measurable diuresis during the first 4-6 hours in normally hydrated subjects. The simulation analysis has also revealed that the transcapillary filtration mechanism indicated in Figure 2 is self-limiting as a means of relieving central hypervolemia and may only be important during the first few hours of weightlessness. Thereafter, lymph flow or inward filtration may restore interstitial volume to normal. This self-limiting phenomenon occurs because plasma colloidal concentration increases as fluid moves into the interstitium, which opposes further filtration. The limited data on this subject indeed suggests that interstitial fluid volume remains relatively constant even after a month or more of space flight (Refs. 6, 10). Taken as a whole, these analyses indicate that in the well-hydrated subject, the kidneys can be expected to be the principal avenue of fluid regulation during weightlessness.

Even more important than the above, was that simulation of the mathematical model resulted in clarification of the dynamic nature of the fluid system as it responds to the acute onset of weightlessness. Furthermore, the dynamic behavior, by itself, appears to explain some of the paradoxical experimental results that were being reported. This is illustrated most clearly in a computer simulation of the first 24 hours of hypogravity (Refs. 1, 11, 12) shown in Figure 4. The validity of these model responses is apparent from the close agreement to the head-down tilt studies conducted by Blomqvist and co-workers (Ref. 13). During the first several hours the mathematical model predicts a number of changes in fluid volumes, hemodynamics, and renal-endocrine function (i.e., see open circles in Figure 4), all of which are in essential agreement with the hypothesis diagram of Figure 1. If measurements were taken at any time later than that (i.e., see filled circles in Figure 4), the model predicts that one may find hormone levels elevated (angiotensin, aldosterone, ADH), renal excretion stabilized or reduced, or venous pressures below normal. Indeed, such "paradoxical" findings have in fact been observed in space flight (Refs. 9, 14, 15). However, the simulation analysis suggests that these results are merely the normal response of a highly complex feedback system which has succeeded in correcting the central hypervolemia at the expense of a stabilized reduction in plasma and leg volume. In fact, except for

fluid volume changes, all other relevant variables examined exhibit a transient biphasic behavior, displaying, in some cases, an overshoot phenomena. These model results strongly suggest that if confirmation of the fluid redistribution hypothesis is to be obtained, then the measurements should be conducted in well hydrated individuals within a few hours after launch. By the time the fluid volume decrements can be measured, it may be too late to capture the events which caused these losses.

In summary, a modeling analysis of the fluid-shift hypothesis has revealed that the hypothesis is based on concepts of acute fluid regulation, specifically, those which are related only to volume and pressure disturbances. In the real system, there are both short-term and long-term control mechanisms. Therefore, the fluid-shift hypothesis pertains only to a static slice of time, during the first few hours of weightlessness, while the real system is actually in dynamic motion.

For the most part, a reasonable understanding currently exists regarding the possible mechanisms of acute fluid disturbances in space flight (although most of the data is derived from ground-based experimental models). What is needed is an equally broad theory of long-term fluid, electrolyte and hormonal regulation to help explain the large data base accumulated from bed rest and space flight studies of weeks and months in duration. The following sections describe several different problem areas of long-term control during space flight, and suggest possible mechanisms derived from a systematic simulation study utilizing the mathematical models. These aspects are concerned with long-term control of blood volume, venous pressure, and hormone secretion.

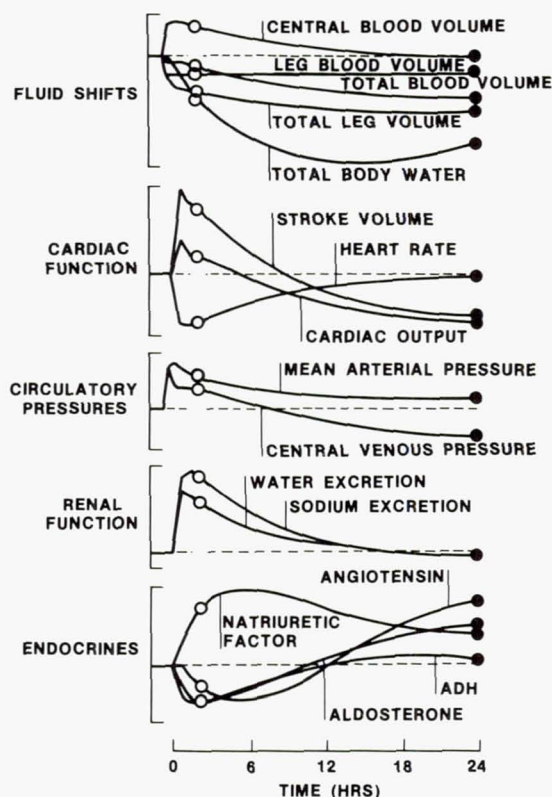


Figure 4. Simulation of head-down tilt (-6 degree)

4. BLOOD VOLUME REGULATION

A typical finding in astronauts returning from space flight is a reduction in total circulating blood volume of about ten percent (Ref. 16). Although not measured directly during flight, this blood volume reduction is believed to be the result of an acute plasma volume loss, followed by a more gradual loss of red cell mass, a finding commonly reported during bed rest (Ref. 17). Figure 5 illustrates the data for blood volume changes reported for the three Skylab missions (Ref 10). Several important questions related to plasma and blood volume regulation were subjected to analysis using model simulation. In particular, what determines the extent of plasma volume loss? What factors are involved in the loss of red cell mass? And, how is long-term regulation of total blood volume accomplished in space flight?

As discussed in the previous section, the etiology of the plasma loss is most likely a direct response to headward fluid shifts (i.e. central hypervolemia). Two different types of fluids, blood and interstitial filtrate, are shifted cephalad during space flight. Using computer simulation techniques it is possible to distinguish between the separate effects of blood shifting from the legs versus tissue filtrate shifting from the legs during the onset of weightlessness. Figure 6 summarizes the results of two model simulations in which 500 ml of either blood or filtrate from the legs were forced from the leg blood vessels or from the leg tissue compartment, respectively. Once this somewhat artificial initiating maneuver was completed (in a matter of minutes), the fluids distributed themselves in a manner consistent with hemodynamic factors.

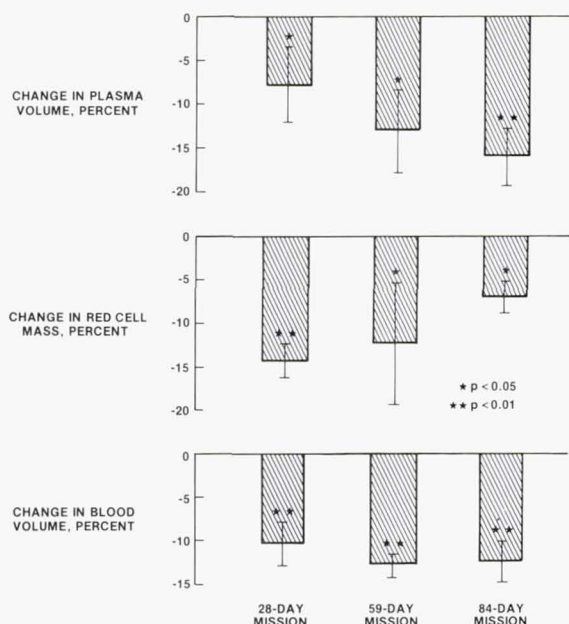


Figure 5. Blood volume alterations in Skylab crew

The top panel of Figure 6 indicates the rate at which equal amounts of these two types of fluids were shifted from the legs. Although most of the responses shown in Figure 6 differ for the two fluids, the disparity in the response of the blood volume compartment is perhaps the most dramatic. For the case of blood shifting from the legs, the

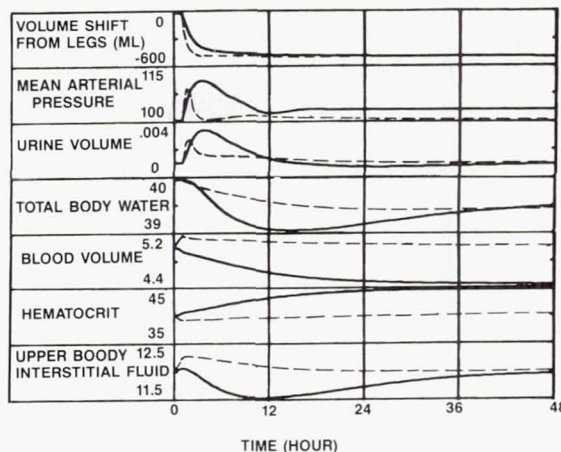


Figure 6. Blood vs. filtrate shift from legs
500 ml blood shift from legs (———)
500 ml filtrate shift from legs (----)

blood volume decreases over time (as we have come to expect for hypogravic maneuvers). Conversely, when leg filtrate shifts in the circulation, the blood volume first increases and then returns to control levels, but never falls below control. (The hematocrit responses merely mirror the blood volume responses because red cell mass is reasonably constant). This seems to indicate that the blood volume decrease in hypogravity is ultimately a result of blood shifting from the legs and activating the renal pathways (Figure 3), which in turn relieves the upper body volume disturbance. Leg filtrate, on the other hand merely enters the vasculature in the legs and exits the vasculature in the upper body (either to the kidneys or temporarily into the tissue compartment). Notice that the model predicts that in the case of blood shifts, the blood volume is depleted by about 500 ml, an amount similar to that which was originally shifted from the legs.

It may be possible to speculate from these studies that the blood volume loss in space flight is a result of, and similar in magnitude to, the shift of blood from the peripheral circulation in the legs toward the upper body. In terms of control theory, the set-point for blood volume regulation has been changed or reset. Removal of interstitial fluid from the legs contributes to overall body dehydration but not to blood volume loss per se. Furthermore, as predicted in Figure 4, the depletion of blood volume (and possibly stress relaxation of the large veins) might restore the expanded central volume and elevated pressures to their preflight levels. In other words, fluid congestion of the upper thoracic cavity (not necessarily including the head and neck tissues in which some excess fluid presumably remains) is predicted to be a short-lived phenomena in space flight. This prediction is supported by recent findings of reduced venous pressures in astronauts (Ref. 14).

The failure of plasma volume to return to normal during prolonged flight (even with a normal and ad libitum fluid intake), as noted in Figure 5 is presumptive evidence of the active involvement of blood volume controllers and perhaps an effective change in volume set-point. Thus, although an expected diuresis has not yet been observed early in a flight, volume receptors and renal excretion pathways may be continually responding to the tendency for fluids to pool headward, thereby acting

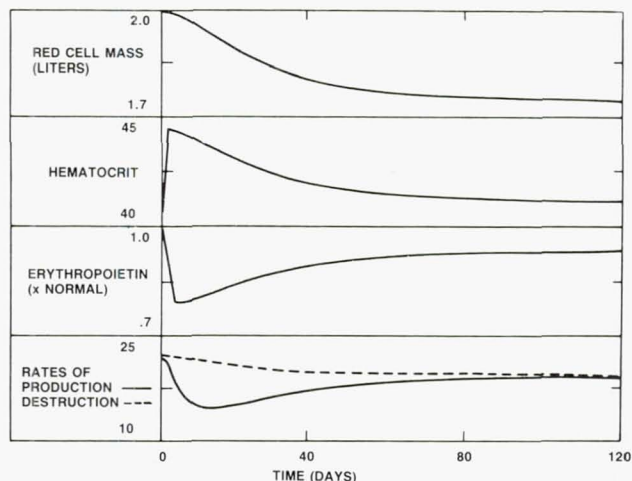


Figure 7. Response to a step decrease (300 ml) of plasma volume

to maintain a reduced blood volume. This hypothesis could be tested by administration of a fluid load in space flight. The model predicts that any fluid load would eventually be excreted and the plasma volume would ultimately be maintained at the reduced levels consistent with the effective change in total blood volume set-point.

The blood volume changes noted above primarily reflect the plasma component of blood. An important question concerning blood volume regulation in weightlessness is the fate of red cells. As is well known, the red cell mass almost invariably declines in bed rest and space flight. The cause has not yet been established (Ref. 16). An extensive modeling effort of the erythropoiesis system has provided additional insight into the nature of the observed red cell mass changes (Refs. 4, 17, 18). Several possible mechanisms for the "anemia" of space flight were suggested by simulation analysis of the model of erythropoiesis regulation. These include changes in renal blood flow, alterations of oxy-hemoglobin affinity, reduction of plasma volume, moderate hemorrhage or hemolysis, and inadequate caloric intake. No single mechanism appears to be solely responsible. One of the more attractive implications of the model analyses is that the hemocentration resulting from plasma loss, a frequent finding in bed rest and space flight (Refs. 16-19), is possibly a major factor leading

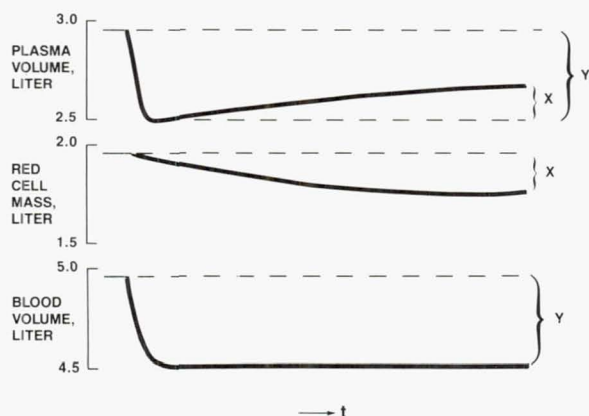


Figure 8.

Relationship between blood volume components during space flight simulation

to the loss of red cell mass. Accordingly, the increase in blood hemoglobin concentration would be expected to enhance oxygen delivery to the tissues, particularly the renal oxygen sensors which are involved in activating the secretion of erythropoietin. An effective overload of oxygen to these renal sites would be expected to suppress erythropoietin levels, which in turn could dramatically decrease red cell production. Without new red cells entering the circulation, the normal attrition of erythrocytes would begin to deplete the red cell mass. This process could continue until rates of red cell production and destruction equilibrate (see Figure 7). This scenario is based on the plausible concept that red cell production in space, as on earth, is regulated by a balance of oxygen supply and demand.

An equally intriguing and unexpected observation from the modeling study is summarized in Figure 8. A simulation of hypogravity was achieved by the same head-down tilt maneuver that was employed for the study shown in Figure 4. The rapid fall in plasma volume was caused by the mechanisms described in Figures 2 and 3. The more gradual depletion of red cells resulted from the hemocentration mechanism described above. The model predicts that blood volume will be maintained constant at a new and reduced level, consistent with the concept of a change in set-point. As a result, the plasma volume (which is free to change in the Guyton model) begins to slowly recover from its minimum value at a rate which exactly replaces the volume equivalent of red cells which are lost. Although highly speculative, this hypothesis is supported by the data shown in Figure 5. In spite of the variability of plasma volume and red cell mass among the three missions, the blood volume remains relatively constant. Taken as a whole, this suggests that total blood volume, rather than plasma volume or red cell mass, is tightly regulated. Analysis of other flight data may reveal whether this is a general effect.

5. CENTRAL VENOUS PRESSURE

Closely related to the phenomena of acute fluid shifts is the response of the central venous pressure during hypogravity. A study of disturbances of venous pressure provides another example of how modeling can be used in the scientific process. Central venous pressure assumes importance in the physiological monitoring of fluid redistribution in microgravity because it reflects the status of central blood volume. For example, the time course of the cephalad fluid shift and its subsequent normalization is likely to be revealed by monitoring central venous pressure. However, this fundamental information is still lacking because of the inherent difficulty in directly measuring central venous pressure. Nevertheless, central venous pressure has been measured or inferred in a variety of related situations and this data is summarized below.

The first measurements of the expected increases in central venous pressure as a result of hypogravity were obtained during water immersion studies. Arborelius, et al (Ref. 20) measured a dramatic increase in right atrial pressure (about 15 cm H₂O) ten minutes following immersion. Echt (Ref. 21) duplicated these results and determined that central venous pressure remains elevated for at least three hours. As a result of these studies, some believed that central venous pressure would remain quite high for an indeterminate period of time in

space flight. In fact, there was some concern regarding the possibility of right heart congestion (Ref. 22). It was not until Blomqvist and co-workers (Ref. 13) reported on their head-down tilt studies that it was demonstrated that central venous pressure can behave in a highly dynamic manner, first increasing and then decreasing below control levels. Although the increase in central venous pressure was expected, the magnitude of the increase in head-down tilt was much less than observed in the earlier water immersion studies. In addition, the subsequent decrease below control of central venous pressure was unexpected by most investigators. Only recently, have the first inflight measurements been obtained. Bungo (Ref. 23) measuring echocardiographic left diastolic volume and Kirsch, et al (Ref. 14) measuring peripheral venous pressure have both inferred decreased venous pressure after six hours and 22 hours following launch, respectively.

The data presented above does not appear wholly consistent among the various studies nor with the prevailing notions of fluid shift hypothesis (Figure 2). The water immersion results showing increased venous pressure are in obvious accordance with the hypothesis that headward shifts of fluid from the legs will expand the central blood volume. In that light, however, the space flight results indicating decreased venous pressure are confusing - especially because of concomitant observations of fluid congestion in the head and neck. Only the head-down tilt studies of Blomqvist and co-workers (Ref. 13) successfully reconciled these sets of measurements. However, no satisfactory explanations for the observed decrease in venous pressure have yet emerged, either for head-down tilt or space flight.

The mathematical modeling of the circulatory responses to hypogravity, including central venous pressure, was first conducted soon after the water immersion studies had been reported (Ref. 24). In the first attempt to simulate supine bed rest, the Guyton model indicated results very similar to those reported by Blomqvist some years later. However, because the prevailing opinion of that day was that central venous pressure would remain elevated in space flight, the modeling prediction that the venous pressure would quickly decline to below normal (see Figure 4) was not given serious consideration. Following the Blomqvist report, however, the modeling of central venous pressure was revisited (Ref. 12) and a description of this more recent modeling study is summarized below.

A closer examination of the Guyton model indicated that only three factors could be responsible for the decline in venous pressure toward normal after an initial rise due to cephalad fluid shifts. These factors are: an increase in venous compliance or stress relaxation, a decline in blood volume, and an increase in total peripheral resistance. All of these adjustments occur in the model following the initiation of a headward fluid shift; they are parts of an automatic feedback response which attempts to normalize an increase in blood pressure. Because of their role in blood pressure regulation, all three factors could be expected to return central venous pressure to normal. However, it was not obvious at first which one or more of these factors were responsible for the decline of central venous pressure to below normal levels. By using special simulation techniques it was possible to isolate the total peripheral resistance as the

most important factor involved in this process.

Additional analyses were performed with the model to isolate the mechanisms which permit total peripheral resistance to increase. In Figure 9, the dynamic behavior of four factors which control total peripheral resistance in the model is shown during a head-down tilt simulation. The response is quite complex, demonstrating the utility of a model in sorting out and integrating this type of behavior. For the first few hours autoregulation causes total peripheral resistance to increase in an attempt to locally shut down an overperfused tissue; viscosity gradually increases because of hemoconcentration; angiotensin responds to a high pressure stimulus by falling in value; and the autonomic behavior reflects baroreceptor input. After 24 hours only the angiotensin and the viscosity effects remain as stimuli to explain the sustained increase in total peripheral resistance. These model changes can obviously be translated into an hypothesis for experimental testing.

The ability of viscosity (hemoconcentration) to control total peripheral resistance was carefully examined as the simulations shown in Figure 10 indicate. In this figure, simple infusions of similar volume are performed with either plasma or whole blood. The responses of blood volume and arterial pressure are nearly identical in each case, although the hematocrit response is quite different. Also, the hematocrit and total peripheral resistance responses are parallel for each infusion, reasonably suggesting that the former variable is controlling the latter. Furthermore, the response of whole blood infusion appears very similar to the response for head-down tilt (Figure 4) except for the blood volume which, of course, is elevated for the case of infusion and depressed for the head-down tilt case. This similarity is predictable if one thinks of head-down tilt as an autoinfusion of blood from the legs to upper body. In that case, the blood volume response to whole-blood infusion (Figure 10) is analogous to the central blood volume response of head-down tilt (Figure 4). Even more important and germane to this discussion is the fact that venous pressure declines much further below control in the simulation of whole blood infusion compared to the plasma infusion. This demonstrates that a decreased venous pressure is possible without postural change, merely by creating a hemoconcentrated

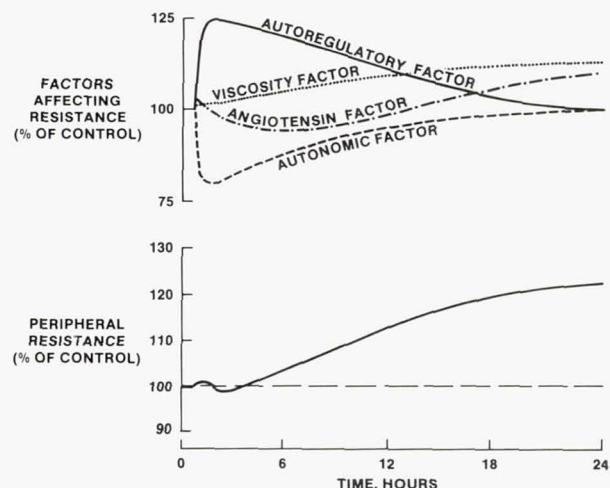


Figure 9. Factors controlling peripheral resistance during head-down tilt

condition. Put more simply, any mechanism which causes total peripheral resistance to increase will tend to lower venous pressure. Although this relationship is known, it is proposed here as the operative mechanism underlying the venous pressure changes observed in space flight.

As a partial validation of this hypothesis, it was possible to compute total peripheral resistance during head-down tilt using Blomqvist's data for arterial and venous pressure and cardiac output. Values for resistance are in agreement with the model with regard to direction, magnitude and temporal relationships. Also, the recent echocardiographic data of Bungo (Ref. 23) indicates an increase in peripheral resistance during space flight. Other experimental studies will be needed, however, to conclusively link changes in resistance with venous pressure and to determine if other factors such as venous compliance can contribute significantly to the decline of venous pressure. The currently proposed experiments for Spacelab on humans and rats will test these relationships (Ref. 25). For periods of time longer than can be studied in the normal one-week Shuttle flight, the model predicts that the elevated peripheral resistance will decline toward baseline levels thereby permitting venous pressure to return towards its baseline as well. Testing this hypothesis will require the long exposure times to weightlessness expected for space station inhabitants.

6. HORMONE REGULATION

A group of renal-regulating hormones consisting of anti-diuretic hormone, aldosterone, and angiotensin, has been the focus of many space flight related studies (Refs. 13, 26, 27). These hormones regulate the ionic concentration of body fluids, particularly, the concentrations of sodium and potassium. A knowledge of inflight hormone disturbances should therefore, provide insight into the status of the fluid-electrolyte and renal systems. However, the findings from space flight have been difficult to interpret or to reconcile with endocrine data obtained from one-g analogs of weightlessness.

Previously, we have organized the endocrine data from a number of hypogravic studies, whether per-

formed in one-g or zero-g, into a qualitative, composite description (Refs. 6, 28). That analysis was based on the assumption that various maneuvers such as water immersion, head-down tilt, bed rest and space flight are parts of a time continuum. All these stresses have the common characteristics of an acute reduction in hydrostatic gradients and a resultant headward shift of fluid. Over longer periods of time, these maneuvers lead to reductions in body water, plasma volume, and electrolytes. In this construct, a six hour water immersion study should indicate the early responses to hypogravity, while a one-week bed rest study should provide the later responses, and a 24-hour head-down tilt study would provide intermediate information. Although these assumptions are not strictly valid, they will provide a point of departure for collating a diverse set of data. The results from such an analysis indicate that the acute hypogravic responses (during a period for which comparable space flight data are lacking) for the three hormones of interest lead to suppressed plasma levels, and that this can be explained on the basis of pressure-volume disturbances. However, the longer-term responses and their control mechanisms are much less clear. In the following analysis, a reconciliation of the differences between the acute and chronic responses to hypogravic stresses is attempted.

A schematic description of the factors which influence the three hormones (as they are represented in the mathematical model), reveal that each hormone is responsive to two general types of controlling stimuli: volume disturbances and electrolyte disturbances (Figure 11). For convenience, the stimuli are shown in Figure 11 in the direction that causes each of the hormones to increase in value. The volume stimuli (as reflected by atrial, renal, or arterial pressures) may provide control only during acute disturbances, because of the existence of several types of adaptive mechanisms indicated in Figure 11, and because the volume disturbances are often corrected by efficient volume-regulating mechanisms (Ref. 29). However, the influence of the electrolyte disturbances (as reflected by plasma sodium and potassium concentrations) is not known to adapt over time. Therefore these electrolyte sensing systems provide a more powerful type of long term control than volume sensors.

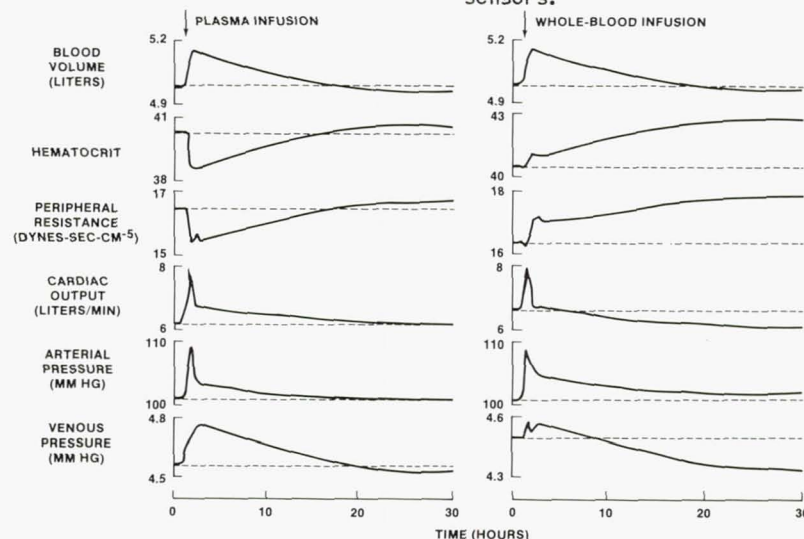


Figure 10. Simulated effect of plasma or whole blood infusion on hemodynamic responses

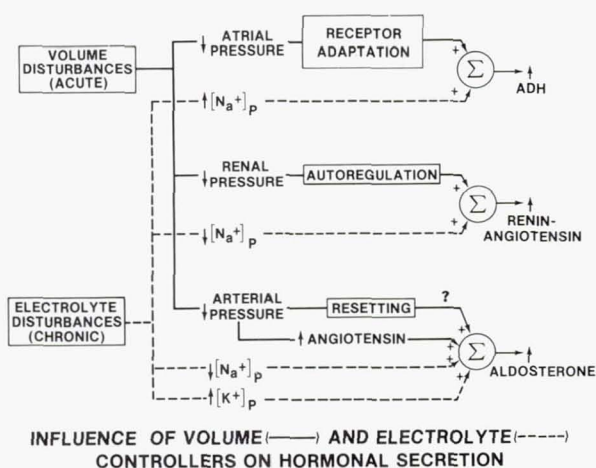


Figure 11.

All of the hormone stimulating factors shown in Figure 11 are known to change at one time or another during hypogravic maneuvers. For example, during the onset of weightlessness, blood pressures are likely to become elevated. Also, plasma sodium is frequently reported to be depressed sufficiently to suppress ADH secretion and elevate angiotensin. Plasma potassium levels (which have a much more powerful effect on aldosterone than plasma sodium levels) may vary upward or downward depending in part on muscle atrophy, metabolic intake, and excretion. These various changes, occurring simultaneously, complicate any attempt to perform an integrated analysis of hormone behavior.

An example of how computer modeling may be useful to account for the dynamics of endocrine control during space flight is presented here for the case of ADH changes (Figure 12). According to the algorithms embedded in the Guyton model, ADH is assumed to be under dual control of atrial (venous) pressure and plasma sodium concentration. The following assumptions are made regarding these stimuli under weightless conditions as shown in Figure 12: a) the central venous pressure response is similar to that found during head-down tilt; b) hyponatremia develops within several days; and c) ADH is primarily pressure/volume sensitive during the acute phase which may last until pressures begin to decline toward control levels, after which time the sensitivity gradually shifts to favor osmo-control due to some form of volume-receptor adaptation or pressure normalization. That ADH is primarily responsive to chronic osmotic changes in space flight is also consistent with the view that this hormone is a powerful long-term controller of plasma osmolality (Ref. 29).

Although based on several untested assumptions, this hypothesis appears to explain the average ADH response as measured in the Skylab crew during the first month in space (see Figure 13). The complex triphasic response of ADH predicted by the model appears to be reflected by the space-flight data. Specifically, an early decrease in ADH during the first flight day (when ADH was not measured) can be inferred from water immersion studies, head-down tilt studies, and the first postflight day. Similarly, ADH is diminished during the second half of the first month in space. Between these two points in time, ADH may be in transition due to competition between venous pressure and osmo-

control. Computer analyses of aldosterone and angiotensin indicate similar complex waveform responses for all three renal-regulating hormones (Refs. 28, 30). In the light of this hypothesis it is proposed that the long-term behavior of ADH, aldosterone, and angiotensin in space flight can be explained as chronic adaptation to metabolic factors.

An additional model prediction that remains to be confirmed is the existence of a natriuretic hormone (Figure 4) which responds to blood pressure disturbances and which can produce elevated levels of urinary sodium and hyponatremia in spite of elevated aldosterone levels. The natriuretic factor was added to the Guyton model for the primary purpose of simulating long-term hyponatremia. No other plausible change to the model was successful in producing this condition without creating adverse responses in other related systems. Without the presence of the natriuretic factor, the etiology of hyponatremia in space flight remains unresolved and puzzling because aldosterone was elevated and ADH suppressed, conditions that should promote correction of hyponatremia. Although the adaptive value of a hyponatremic extracellular fluid is not clear, hyponatremia is known to activate the renin-angiotensin system, induce hypersecretion of aldosterone, depress thirst, depress ADH secretion and promote renal excretion of water, all of which were noted to varying degrees in the Skylab crew. One unanticipated effect of hyponatremia that was revealed, at least in the model system, was a preservation of almost a liter of intracellular fluid. Fluid leaves the cellular compartment as potassium is depleted, and this action is opposed by a reverse osmotic gradient as plasma sodium levels decline, even by a few percent.

The long-term response of renal water excretion during hypogravity is shown for a month-long space flight in Figure 13. In Skylab, evaporative water loss was shown to be diminished by approximately 10

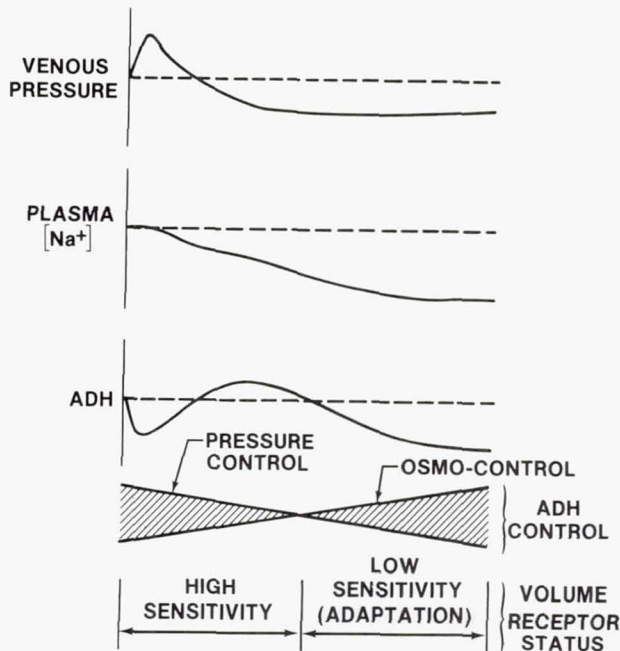


Figure 12. Hypothesis for ADH control during space flight

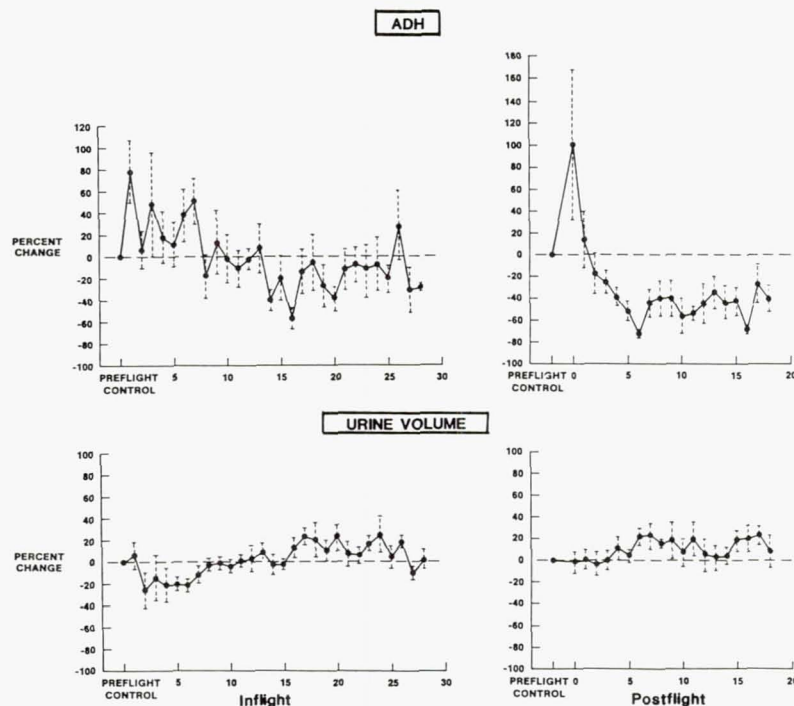


Figure 13. Urinary ADH and daily urine volume shown as percent change from preflight levels

percent (Ref. 31) which can be thought of as an effective increase in net fluid intake. As may be expected in such cases, urine output also increased a corresponding amount. Also, Figure 13 illustrates that on the average, ADH levels and urine volume are inversely related in zero-g, as one would expect from physiological theory in one-g. This has never been previously demonstrated.

These studies suggest a generalized theory that can explain the overall behavior of the major renal-regulating hormones. In particular, there appears to be a shift from short-term volume control to long-term metabolic control, especially resulting from chronic changes in electrolyte disturbances. While the acute affect generally leads to suppressed endocrine levels, the long-term effect can vary widely, depending on the particular combination of metabolic factors. These factors, such as diet, sweat loss, physical activity, and muscle atrophy, can alter the plasma electrolytes and thus influence hormone behavior. The analysis becomes complex because hormone changes then have a feedback effect on the electrolyte levels. Moreover, a transition between volume control and electrolyte control can produce a dynamic multi-phasic endocrine response. Within limits, we have shown that a mathematical model of this system is an effective tool with which to study many of these complex effects.

7. DISCUSSION

7.1 Future Work

Inasmuch as this research effort was concerned not only with specific mathematical models and simulation techniques, but also with the design and interpretation of physiological flight experiments it is natural to consider the future directions in both of these areas. With regard to the tools of modeling, it is clear that the modified Guyton model has served well in studying complex, whole-

body phenomena concerned with circulatory and fluid regulation. However, it is also apparent that the model is limited; it does not contain sufficient detail to allow the simulation of certain physiological behavior. Since the publication of the Guyton model in 1972, there have been advances in several areas which should be considered for incorporation into the model. The current renal subsystem, for example, lacks some of the known intricacies regarding peritubular capillary effects, third factor effects, and factors influencing intrarenal blood flow. Also, acid-base balance and regulation is not represented in the current model. Similarly, modification of the Guyton model's hormone regulating pathways should reflect current knowledge in this area, especially with regard to relative sensitivities of competitive stimuli. Hormones such as natriuretic factor, prostaglandins and norepinephrine should be included in the next model generation. Finally, a more realistic approach to mathematically simulating hypogravity may involve changing the reference position of the model from the supine to the orthostatic position. This would provide an additional degree of freedom so that, for example, it would be possible to more realistically distinguish between supine and head-down bed rest.

From an experimental viewpoint, it is possible to predict more rapid advances in data collection and hypothesis testing than has been witnessed in the past. No longer are observations limited only to pre- and postflight measurements, to non-invasive measurements, or to studies on only a few crewmembers. The most pressing need in the fluid-electrolyte discipline is the information related to acute changes. Virtually no data has been collected during the first few hours of weightlessness, when many of the responses discussed here may be transiently observed. Important aspects of an acute study should include confirmation of the expected headward volume shifts, the suppression and rebound of the renal-regulating hormones, the

existence of natriuretic factor, blood pressure disturbances, altered renal clearances, and the reduction in plasma volume. These are the type of short-term experiments that could ideally be conducted on the Shuttle and its Spacelab.

Studies of longer term adaptation, such as those conducted on a space station, could address such metabolic issues as the control of renal regulation, the etiology of hyponatremia, the factors leading to space flight anemia, the degree of long-term fluid congestion of the upper body, confirmation of sweat suppression, and the basis for increased systemic flow resistance. Other experiments are required to study the influence of diet as a potential countermeasure, shifts of circadian cycles, direct measurements of basal and work energy utilization, and potential metabolic changes at the microcirculatory and cellular levels. The role of hormonal regulators of renal functions during the chronic or adaptive phase of flight is not yet clearly defined. An assessment of renal function would be possible by performing renal stress tests in humans (fluid loading, salt loading, dehydration) and from studying glomeruli and tubular dynamics by renal micropuncture techniques in animals. Much of this information should be considered essential for defining a baseline norm for zero-g adaptation and health.

Several comprehensive experiments, on humans, primates, and rats have recently been selected for flight in answer to some of these research needs (Ref. 25). They will address fluid, electrolyte and circulatory issues including direct measurements of body fluids, venous pressure, renal regulating hormones, renal function, microcirculatory changes and circadian rhythms. A number of these studies resulted from the conclusions generated by the mathematical modeling analyses, some of which have been presented in this paper.

7.2 Conclusion

The findings and hypotheses discussed in this paper with regard to the fluid-electrolyte discipline have been integrated into a larger hypothesis of space-flight adaptation (Refs. 1, 6, 32, 33). All of the modeling studies conducted to date indicate that a significant portion of the known responses to weightlessness can be explained in terms of normal, although complex, feedback-regulatory processes. It appears however, that the feedback mechanisms that are known to explain the acute effects of space flight may not be capable of accounting for the longer term effects. In fact, little is known of the long-term adaptive mechanisms which occur in weightlessness and it is possible that models such as those used here can play an important role in suggesting testable hypotheses. In this context, models have proven to be least effective when used to merely predict a physiological outcome, but have been most effective when they are used to probe the behavior of the system and identify the critical questions.

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SESSION VII

SUMMARY

The Chairman, Dr. R.J. White of NASA headquarters, introduced the session by discussing the notions that the kinds of questions which a science asks is one fundamental characterisation of the science itself, and that the use of mathematical modelling in any science expands, in a real way, the class of questions which may be asked. When used in the appropriate spirit, Dr. White pointed out that mathematical models can alter the fabric of a science by changing the nature of the questions asked within that science. This has been demonstrated over and over again in many different disciplines, and we are beginning to see the results of using models in the area of gravitational physiology.

Following Dr. Joel Leonard's invited paper on the subject of mathematical models, Dr. R.J. Cohen commented on the importance of the use of simple models in discussing particular problems. He gave some examples of simple models he has used to examine disturbances in the cardiac system, and included in these examples a computer demonstration of a cardiac model which could be used to simulate the phenomenon of fibrillation.

Dr. M. Droulez pointed out the difficulty of modelling certain systems that are really complex, such as the vestibular system and its associated postural control systems, where even simple tasks, such as postural variation while holding a glass of water, involve complex pathways that are incompletely understood.

Dr. V. Gurfinkel noted that it is sometimes useful to combine mathematical and mechanical models. He discussed the control of movement in a six-legged robot complete with tactile senses and artificial otoliths. The development of a mathematical bio-feedback-based, control system for this mechanical robot can contribute much to one's understanding of physiological processes.

Dr. P. McCormack discussed the importance of including an appropriate model of the gastrointestinal tract in any fluid and electrolyte model concerned with space flight, particularly as the uptake of materials by the body is likely to be altered in space. He provided a sample model to illustrate his ideas.

Dr. L. Young (substituting for Dr. C. Oman who was unable to attend) pointed out that every model, in order to really be valuable to science, must contain the seeds of its own destruction. That is, models are made to be refuted by data and replaced by other, stronger, models. Dr. Young also remarked that mathematical modelling is not an optional technique to use in conjunction with physiological systems. Such systems are so complex that they require mathematical models if any kind of basic explanation of the system's behaviour is to be developed.

Dr. M. Patat stated that his particular interest in models was related to his need to interpret the full meaning of echocardiographic data gathered in space. He noted that he personally was new to modelling and that he had questions, not explanations, at present.

Finally, Dr. John Rummel reviewed the historical context for the use of models to study problems of gravitational physiology. Such modelling was supported by NASA in the early 1970's to help in the interpretation of data from the Skylab missions, and, in retrospect, it was a highly significant step in the analysis process. Dr. Rummel then pointed out that one early development, that of what was termed a 'whole-body algorithm,' was, and still appears to be, ahead of its time. This model represented an overall model of many of the major human systems, including the cardiovascular system, fluid and electrolyte system, respiratory system, and thermoregulatory system, integrated together to form a physiological model of general applicability.

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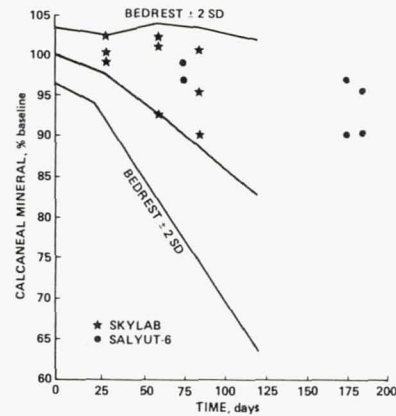
SPACEFLIGHT AND CALCIUM METABOLISM

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Calcium metabolism data from spaceflight have been obtained primarily from Skylab astronauts, from Soviet Salyut-6 cosmonauts, and from growing rats flown either on the Soviet Cosmos series or Spacelab 3. In this report, the results from Skylab astronauts will be compared to data from bedrested subjects, and the results from Cosmos rats will be compared to data from a ground-based rat model, to help explain 1) how spaceflight or gravitational unloading alters calcium metabolism in adult humans and growing rats, 2) the relevance of the observations of bone dynamics in growing rats to the changes in adult man, and 3) the sequence of events leading to changes in calcium metabolism during spaceflight. An hypothetical scheme of the mechanisms causing altered bone mass during spaceflight will be proposed.

Calcium metabolism encompasses two systems; one system regulates calcium homeostasis (defined as regulation of mineral metabolism) and a second system controls bone homeostasis (defined as maintenance of bone structural integrity). The regulation of bone structural integrity by mineral metabolism has been studied in greater detail and is more readily comprehended than the influence of local bone factors and biomechanical forces on calcium homeostasis. The calciotropic hormones (parathyroid hormone [PTH], calcitonin [CT], and the active metabolite of vitamin D [$1,25(\text{OH})_2\text{D}_3$]) interact with bone cells, kidney, and gut to assure that blood calcium is closely regulated and that ionic calcium is adequately distributed for bodily functions. Alternately, changes in bone architecture or metabolism large enough to alter calcium exchange and serum calcium can be expected to trigger responses in the calciotropic hormones and change the flow of calcium through the intestine and kidney which, in turn, changes bone cell metabolism. During spaceflight, the entire skeleton (which contains 99% of the calcium in the body) is probably involved (15,18); however, we do not know if changes in calcium metabolism reflect primarily alterations in bone or calcium homeostasis, but an interdependence of these two regulatory systems obviously exists.

Bone mass in both bedrested subjects and crewmembers was measured using the same technique (22). No change in radius or ulnar density has been reported in adult humans during either bedrest or spaceflight, but this sampling site measures predominately cortical bone (which has a very slow turnover rate in adult humans) (3, 22). Trabecular bone has a higher turnover rate than cortical bone; changes in trabecular bone mass, estimated as calcaneal or heel-bone mineral density during bedrest and spaceflight, are depicted in Fig. 1. The average decrease in bone density at this sampling site in bedrested subjects exceeds the change in astronauts or cosmonauts. Little or no calcis mineral loss is observed during the first month in either group. Loss in space amounts to only about 7% after 184 days or a projected rate of loss of approximately 1% per month while bone mineral appears to be lost at a rate of about 5% per month during bedrest. Possible explanations for the different rate of loss between



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Fig. 1. Changes in calcaneal mass during flight as compared with bedrest (22, 17). The shaded area represents ± 2 S.D. from the mean of bedrest changes. The solid middle line represents the mean value from 15 bedrested subjects. These bedrest data are only given for 120 days although the studies lasted 6-9 months (22). For all subjects, calcaneal mineral mass is expressed as a percent of the baseline value taken prior to bedrest or flight.

bedrest and spaceflight include age, exercise, and gravity. Bedrested subjects were at least a decade younger than crewmen; bone turnover rates decrease with age, thus young adults have higher rates making their bones more vulnerable to metabolic changes. Exercise is usually forbidden during bedrest. The amount and type of exercise performed during spaceflight is shown in Fig. 2. The combined use of the bicycle ergometer, MK-1 and 2, and the treadmill on Skylab 4 did minimize loss of muscle mass and strength (18); perhaps this type and amount of exercise impede loss of bone mass also. The Soviets use similar exercise regimens (17). If bone loss during spaceflight is retarded by exercise, then we may not be able to determine adaptation of the human skeleton to spaceflight without inflight skeletal loading. Ethical and practical concerns for crewmembers may make us dependent upon animal models to investigate adaptation to the space environment uncompromised by exercises which provide some skeletal loading. However, even with exercise some bone loss does occur during spaceflight.

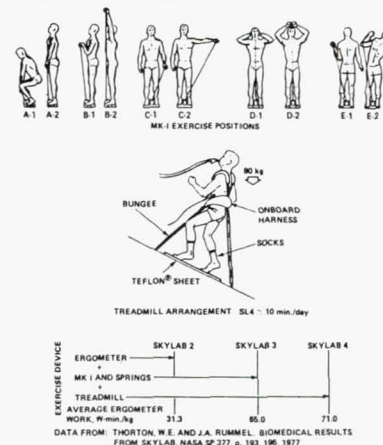


Fig. 2. Exercise equipment and average ergometer work during the Skylab series (18).

We do not know whether bone loss will continue throughout longer missions. However, we do have suggestions from bedrest (21) and from paraplegic patients (11) that trabecular bone loss may abate after about 6 months. Fig. 3 shows individual data from the Donaldson bedrest study which lasted 225 days (21). Decrease in density is essentially linear until about 180 days when it tends to plateau in 2 of the 3 subjects. Minaire et al. (11), found indications of a similar time-related adaptation in trabecular bone volume in paraplegic patients. We do not know if similar adaptation occurs during spaceflight; if gravity is responsible for resetting bone metabolism, then bone loss during spaceflight could proceed indefinitely. Changes in bone cell activity during gravitational unloading were found in iliac crest biopsies analyzed by Jowsey (9) from bedrested subjects and Minaire et al. (11) from paraplegics; an immediate and dramatic decrease in bone formation with indications of a net increase in bone resorption were reported.

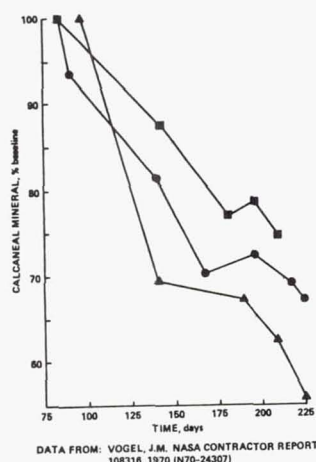


Fig. 3. Calcaneal mineral from bedrested subjects (21). Measurements were not begun until about the third month of the study and the first measurement was used as a basis for comparison of all other measurements. Note break in curve at about 6 months.

Paradoxically, smaller changes in skeletal homeostasis in Skylab astronauts cause larger alterations in calcium homeostasis than the more pronounced changes in skeletal homeostasis in bedrested subjects. As shown in Fig. 4, significant increases in plasma calcium occur within the first 3 days of spaceflight and persist in the high normal range throughout the longest mission (84 days) whereas no significant changes are seen during bedrest. Comparison of these results suggests that normalization of serum calcium through endocrine regulation of calcium transport in bone, kidney, and gut occur as expected only in bedrested subjects. Calcium intake (Fig. 5) was approximately 900 mg/day in the prestudy period; it increased during spaceflight due to higher caloric intakes associated with exercise. Interestingly, fecal calcium excretion more closely approximates dietary calcium during the second month of study (Fig. 5) in both experimental situations. Fecal calcium data from bedrest or spaceflight show no dramatic change from prestudy levels, but small significant differences could be obscured by the large time periods averaged in these plots.

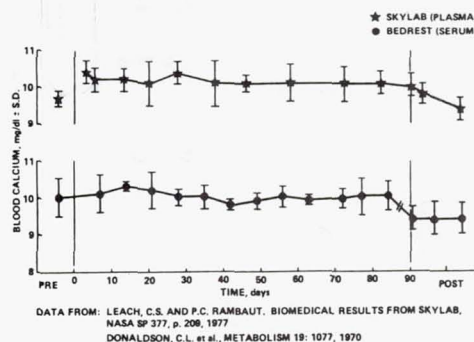


Fig. 4. Blood calcium data from Skylab (10) and the Donaldson bedrest study (3). The shaded area represents the normal control range as reported by Mayo Clinic.

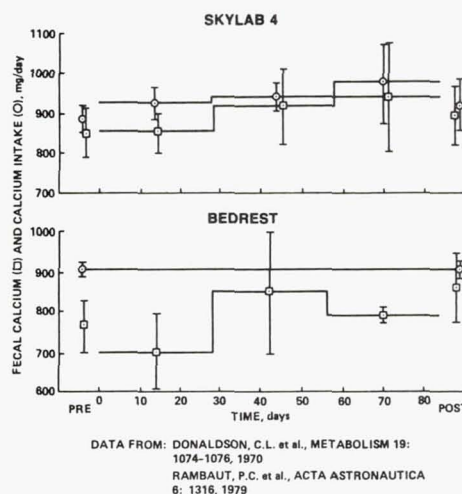


Fig. 5. Calcium intake and fecal calcium data from Skylab 4 (13) and the Donaldson bedrest study (3).

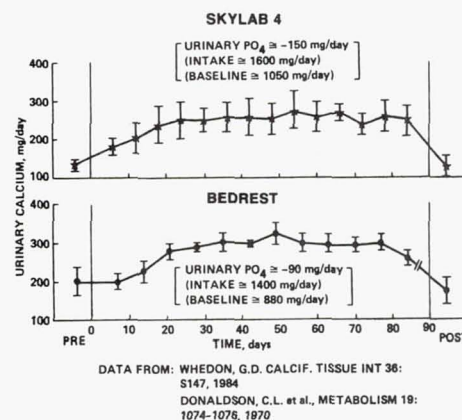


Fig. 6. Urinary calcium data from Skylab 4 (19) and the Donaldson bedrest study (3). Urinary phosphate was also increased in both experimental conditions and negative phosphate balance occurred.

Urinary calcium excretion during Skylab 4 (19) and bedrest (3) show similar changes which are depicted in Fig. 6. During flight, urinary calcium appears to increase more rapidly than during bedrest, but the increase to 1.5 to 2 times baseline values occurs in both groups. Unlike

bedrest calciuria which starts to decrease after about 80 days, urinary calcium excretion during spaceflight shows no sign of decreasing. Calciuria accounts for most of the negative calcium balance during spaceflight. Grigoriev (6) observed exaggerated calciuria and calcemia in cosmonauts who were given an oral calcium lactate load after spaceflight. The persistent calciuria during flight and the hypersensitivity to an oral calcium load postflight is reminiscent of hypothyroid patients who not only have reduced CT levels, but also have reduced bone turnover (1).

Regulation of calcium metabolism during spaceflight appears to be different than during bedrest. In particular, spaceflight is characterized by slower loss of trabecular bone mass, by persistent and stable increase in serum calcium, and by earlier and more sustained calciuria than bedrest. Evaluation of the calciotropic endocrine status and measurement of bone turnover in crewmembers during flight is critically needed to further evaluate these differences.

Skeletal metabolism and the internal architecture of bone in growing rats are very different from adult humans, but the metabolism of individual bone cells (i.e., osteoblasts and osteoclasts) probably does not differ greatly among species. Important data have been gathered from growing rats aboard the Soviet Cosmos biosatellite missions, but these data must be interpreted with an awareness not only of species differences, but also of markedly different rates and directions of bone cell dynamics. As illustrated in Fig. 7, bone is constantly being both shaped and increased in length and diameter in growing animals; osteoblasts (bone forming cells) and osteoclasts (bone resorbing cells) are present simultaneously, but on different surfaces. Growth applies to the activity in the growth plate region which causes elongation of bone from cartilage anlagen while modeling denotes the sculpting processes that follow elongation of bone (4). Both events occur simultaneously in normally growing animals and determine the size and shape of each bone; growth and modeling are collectively referred to as modeling in this paper. Formation exceeds resorption in the modeling skeleton but formation and resorption rates are much higher in the modeling skeleton than in the remodeling (adult) skeleton. Formation is the driver in the modeling skeleton. In the remodeling skeleton, formation and resorption are usually in equilibrium. When a stimulus directs adult bone to renew mass or repair defects, osteoclasts are attracted to the appropriate bone surface or interior and chew away part of the bone. After the osteoclasts finish their activity, osteoblasts appear on the same bone surface to fill in the resorption cavity. The activation-resorption-formation cycle described by Frost (4) determines the number of active bone remodeling foci at a given time. The period of time between initiation and completion of resorption and formation foci is 3 to 6 months in adult humans. The cellular driver in the adult skeleton is the osteoclast.

Bone experiments on growing rats from the Soviet Cosmos biosatellites have contributed significantly to our knowledge of the effect of spaceflight on the modeling skeleton. Histomorphometric analyses of shaft or cortical bone (20) suggest that cessation, rather than suppression, of bone formation may occur. An hypomineralized arrest line (16) suggests that mature matrix does mineralize, but that matrix which had not matured

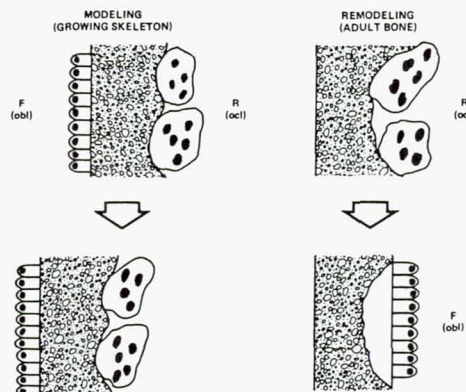


Fig. 7. Artist's concept of bone cell activity and sequence in the growing (modeling) and adult (remodeling) skeleton. F is formation, R is resorption, obl is osteoblast, and ocl is osteoclast.

prior to flight does not mature normally (15) and, hence, does not mineralize. Sampling sites ranging from jaw (15) through rib (20) and long bones (20) show at least some suppression of bone formation suggesting that bone changes in the modeling skeleton are not limited to weightbearing bones. Interestingly, bone surfaces without muscle attachment appeared to be more severely impacted by spaceflight than surfaces with muscle attachments (15,16). Analyses of trabecular bone (8) revealed significant decreases in the mass of mineralized tissue and a decrease in the osteoblast population immediately adjacent to the growth cartilage-metaphyseal junction; marrow fat increased at this sampling site, but osteoclast numbers were unchanged. Using stable isotopes, Cann and Adachi (2) directly measured bone calcium exchange and reported that bone resorption was essentially unchanged. They also showed that calcium excretion did not change during spaceflight eliminating fecal and/or urinary calcium as the major source of calcium loss in these animals. Pitts et al. (12), reported a 17% decrease in total body calcium and, hence, a 17% decrease in total bone mineral. Bone alterations did not immediately return to normal following flight, and formation rates appeared to take about 3 days to return to normal suggesting that progenitor cells had to be stimulated to differentiate to osteoblasts before matrix was produced; these data are consistent with the osteoblast histogenesis sequence established by Roberts (14). Preliminary data from Spacelab 3 are contained in this issue of the Physiologist and suggest that significant changes in bone cell function and metabolism in growing rats do occur within 8 days of spaceflight.

Data obtained from a rat model simulating certain aspects of space flight suggest that changes in bone formation, but not resorption, and bone metabolism do occur but are transient (5). Decreases in the serum concentration of the active metabolite of vitamin D, $1,25(\text{OH})_2\text{D}_3$, occur on this model but have been shown to be secondary to the changes in bone (7), since constant infusion of this vitamin D metabolite did not alter the bone changes.

An hypothetical scheme to explain spaceflight alterations in calcium metabolism has limitations posed not only by species differences, but also the following considerations. 1) Adult bone data reflect a skeleton where resorption precedes and directs formation, whereas the growing skeleton

reflects continuous formation and resorption with formation predominating; both formation and resorption rates are much higher in the growing, than in the adult, animal. 2) Time in flight is limited to few subjects who cannot be sampled with any frequency. 3) Exercise during flight may minimize changes in the musculoskeletal system. 4) Gastrointestinal absorption does not appear to change dramatically in either adult humans or growing rats during spaceflights of 84 or 19 days, respectively. 5) Fluid shifts have been noted to occur in both humans and rats during flight, but whether these changes participate in or cause the bone changes is not established. 6) Urine samples have been collected inflight from humans but not rats. Urine/fecal pools were collected from rats aboard Cosmos 1129. 7) Bone measurements to date have occurred postflight.

Given the preceding qualifications, we propose that the primary effect of spaceflight is directly on bone cell metabolism. Indirect effects on skeletal homeostasis mediated by fluid shifts cannot be excluded and may be equally important in explaining bone loss. However, we propose that the first event of gravitational unloading is suppression of bone formation. Net bone resorption may increase. Changes in bone cell activity would lead to the second event which is projected as an increase in blood calcium which in turn would initiate a cascade of effects of the calciotropic hormones. The third series of events would include a decrease in PTH and an increase in urinary calcium directly related to the increase in serum calcium. The fourth series, responding to the decrease in PTH, would include decreased production of the active metabolite of vitamin D and an increase in urinary calcium. The theoretical decrease in $1,25(\text{OH})_2\text{D}_3$ should cause the fifth and final event which would be a decrease in intestinal calcium absorption. This speculative scheme is based on our concepts of calcium metabolism on earth which may not be operative during spaceflight. Insensitivity of some, but not all, calcium regulating systems may be a basic problem. Many critical data points are missing. A lack of gravity requires less structural support and, hence, less skeletal mass and turnover. During prolonged spaceflights, the gut and kidney could become the major regulators of calcium metabolism. Although such adaptation is appropriate for flight, it could prove a hindrance to readaptation to earth's gravitational field.

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Printed in Switzerland**Gravity - Diving**Dejours (ed.), *Comparative Physiology of Environmental Adaptations*, vol. 2.
8th ESCP Conf., Strasbourg 1986, pp. 15-26 (Karger, Basel 1987)**Gravitational Force and the Cardiovascular System¹***D.R. Pendergast, A.J. Olszowka, M.A. Rokitka, L.E. Farhi*Hermann Rahn Laboratory of Environmental Physiology, Department of
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Over millions of years man has evolved from a water breather in a weightless environment to an air breather in a 1-G environment. This gradual evolution allowed the development of structural and functional changes in both the respiratory and cardiovascular control mechanisms that allow man to cope with specific stresses in his normal habitat. In the area of cardiovascular performance, we have come to rely heavily on complex feedback responses to cope with postural changes, which alter the body axis along which gravitational forces act. The activities of daily living evoke these reflexes as we stand, sit, lie down, or become immersed. Over the past several years, many individuals have 'returned' to a weightless state during space missions whose duration has ranged from a few hours to several months. Missions related to space are likely to increase the time of exposure to the weightless condition. However, some of the mechanisms that are operative at 1-G appear to 'fail' when the 1-G load is reapplied following exposure to a period of weightlessness. There is indisputable evidence that, in some cases, the space environment, by relieving gravitational stresses, has permitted adaptive mechanisms to lapse, causing serious problems upon return to the 1-G condition. If man is to function in a space environment on a periodic basis, he must be able to adjust not only to weightlessness but also to the effects of the earth's gravity upon re-entry and, with a view to the future, to the gravitational fields of other planets that may eventually be reached.

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Successful adaptation to the space environment as we know it requires man to perform work. Cardiovascular feedback mechanisms must cope with two stresses, often combined: postural changes combined with changes in gravitational forces, and physical exercise. Appropriate responses to these stresses may be more difficult to achieve after adaptation to the weightlessness of space; in fact, man may not be able to fully adjust to the stress of gravity unless appropriate feedback responses are reinforced continuously during flight. Although many studies have been conducted prior to, during, and after space flight, due to logistic constraints, information to address the problems stated above is insufficient. To compensate for this lack of information, ground-based simulation studies have been conducted by us and others in an attempt to add insight into the problems of the cardiovascular adaptation to alterations in gravitational force.

The purpose of the present paper is to consider cardiovascular responses to changes in gravitational force. Man is ideally suited to his 1-G environment. Although cardiovascular adjustments are required to accommodate to postural changes and exercise, these are fully accomplished for short periods (min). More challenging stresses are those of short-term microgravity (h) and long-term microgravity (days) and of gravitational forces greater than that of earth. The latter can be simulated in the laboratory and quantitative studies can be conducted.

Weightlessness

Acute Exposure

When a person is standing in air, a large volume of blood is pooled in the periphery. This does not present a problem insofar as venous return (VR) is generally sufficient to maintain a stroke volume (SV) which, when combined with increased heart rate (HR), positive inotropic tone and venomotor tone, result in a cardiac output (\dot{Q}) that is sufficient to perfuse tissue to supply needed nutrients as well as to maintain mean arterial blood pressure. These cardiovascular responses are regulated by complex feedback mechanisms that meet the existing demands as well as the imposed stress of physical exercise. A decrease in the gravitational force results in less peripheral pooling, causing a cephalad shift of blood. This occurs when we assume the supine position or in the head down tilt position (HDT) and results in translocation of 200–500 ml of blood to the thorax. A further shift of blood can be accomplished by applying a graded differential pressure

either from below the diaphragm (lower body positive pressure, LBPP) or a graded differential pressure from distal to proximal (water immersion in water of thermoneutral temperature, WI).

During WI to the neck, ~ 800 ml of blood are translocated to the thorax [1]. The result of these graded increases in thoracic volume are increases in central venous pressure (CVP), right atrial pressure (P_{RA}) and end diastolic volume (EDV); typically heart rate and cardiac contractility remain unchanged. The outcome of these primary changes is an increase in stroke volume and cardiac output. An increase in \dot{Q} could result in an increase in mean arterial pressure (\bar{P}_a); however, this is not usually observed during weightlessness. \bar{P}_a does not increase because of a drop in total peripheral resistance (TPR) that is proportional to the increased \dot{Q} . The decrease in TPR is the result of increased blood flow to many vascular beds including skeletal muscle [3, 19].

One of the most striking responses to WI is the development of a diuresis and natriuresis [12]. The diuresis develops rapidly (with 2 h of WI) while the natriuresis develops more slowly, reaching a peak in 3–4 h. An increase in free water clearance is more marked in normally hydrated subjects as compared to hydropenic subjects [4] with little change in glomerular filtration rate or renal blood flow [12].

Gauer and co-workers [14–16] postulated that the diuresis is due to the inhibition of antidiuretic hormone (ADH), induced by stimulation of the left atrial volume receptors resulting from cardiac stretch (increased pressure and volume). The consequences of the diuresis were postulated to be a reduction in plasma volume (PV) and consequently decreased thoracic blood volume and SV. Initial studies supported this postulate [12]; however, recent studies clearly demonstrate that the cardio-renal coupling is not necessarily as tight as once thought [7, 17].

Data for HDT experiments from several investigators are presented in table I along with our data collected in the supine position and during WI. As indicated in table I, CVP, \dot{Q} and SV increased initially during HDT while HR and \bar{P}_a were not changed significantly. After these initial changes, CVP, \dot{Q} and SV decreased along with HR while \bar{P}_a did not change significantly. Although the magnitude of these changes in the supine position was less ($\sim 5\%$) and during WI greater ($\sim 15\%$), the overall pattern of the responses was similar in the three conditions. Most importantly, the initial increase in \dot{Q} is offset by a decrease in TPR as \bar{P}_a did not change. Over the first 2–3 h of either HDT (6°) or WI, HR and SV decreased, the result being a decrease in \dot{Q} . During HDT the CVP decreased to control levels after 5 h, which could

Table 1. Cardiovascular responses to head down tilt (HDT), supine (S), and water immersion (WI)

		Time							
		0 h	1 h	2 h	3 h	4 h	5 h	6 h	7 h
		(seated)							
CVP, mm Hg	HDT	3.9	3.6	4.6	4.4	—	3.8	—	3.8
\dot{Q} , liters · min ⁻¹	HDT	6.5	7.9	7.8	7.2	—	6.9	—	6.9
	S	6.7	7.6	7.1	6.9	7.0	6.7	6.6	6.9
	WI	6.8	8.8	7.4	7.2	7.0	7.0	7.2	7.1
SV, ml	HDT	94	114	116	110	—	106	—	106
	S	93	106	101	101	107	105	101	104
	WI	92	122	107	109	105	107	109	107
HR, beats · min ⁻¹	HDT	69	69	67	65	—	65	—	65
	S	72	72	70	68	66	64	65	66
	WI	74	72	69	66	67	65	66	66
\bar{P}_a , mm Hg	HDT	97	95	101	98	—	94	—	96
	S	89	86	89	87	88	86	87	88
	WI	88	89	90	88	87	86	87	86
PV, %	HDT	100	101	104	106	105	103	104	101
	S	100	101	102	101	100	99	99	99
	WI	100	104	103	100	100	99	99	98

account for the decreases in SV. During HDT, however, Echt has shown that CVP remains elevated for at least 4 h while SV decreases [11]. During WI, the decreased SV must be due to decreased cardiac contractility, presumably due to a decrease in sympathetic tone. Furthermore, PV, after an initial hemodilution, was not significantly less after 7 h of HDT, S or WI.

All three simulated microgravity experiments resulted in increases in cardiac stretch, urine flow and sodium excretion which were accompanied by a lower plasma renin, aldosterone, and ADH; a careful examination of the results indicate that these changes do not necessarily take place in a parallel manner. For instance, in sedentary subjects, the \dot{Q} increases 35–40% during WI with an increased SV; however, within 2 h of WI it returns to control levels. On the other hand, both the diuresis and natriuresis are sustained for 2–4 h during WI. This dissociation between cardio-renal responses to WI was most dramatically demonstrated in endurance-trained athletes. In these subjects, WI initiates a greater increase in \dot{Q} than in sedentary subjects; furthermore, it is sustained longer. Despite the cardiac

response, the renal responses to WI (both diuretic and natriuretic) were markedly attenuated in trained subjects [7]. The latter finding was accompanied by the lack of an ADH response; plasma-renin-angiotensin (PRA) and aldosterone responses remained normal. The dissociation of cardio-renal responses can be further demonstrated by the nocturnal attenuation of renal responses to WI; cardiac responses are unaltered [27]. The lack of a tight coupling of the cardiac-renal-endocrine responses to WI strongly suggests that while the Gauer-Henry mechanisms may play an important role in eliciting renal-endocrine responses to WI, other mechanisms must contribute to the overall outcome. Furthermore, the renal-endocrine responses do not alter PV over 8 h of WI while SV is decreasing to control levels.

Chronic Exposure

In spite of the absence of changes in PV over an 8-hour period, CVP continued to decrease to only 20% of the pre-HDT values during a 6-day HDT study; \dot{Q} and SV did not decrease further [18, 22]. This finding during HDT agrees with data collected over 2–3 days of space flight where CVP and \dot{Q} were not elevated over control levels; there was, however, a significant diuresis and PV decrease [21, 26, 29]. These 'second phase responses' to microgravity apparently contribute to the cardiovascular deconditioning observed after space flights. The exact mechanism of these responses needs further investigation. Apparently after 7 h of microgravity, CVP and \dot{Q} as well as \bar{P}_a and PV are not elevated above 1-G levels. The question remains as to cause of the second phase of responses to microgravity. A partial explanation may be advanced that is related to the hydration state of the subjects during the 2 to 7-day experiments. The hydration state of these subjects is not well-explained; however, in our supine and WI experiments water loss was not repleted. In previous experiments in which water loss was repleted [4, 12], \dot{Q} remained elevated, and the diuresis persisted over a 4-hour period. In these experiments \dot{Q} was sustained at a high level during WI in spite of a decrease in SV. The effect of partial rehydration during microgravity might be to support the cardiac stretch which could lead to the continuation of the diuresis. Assuming that this tissue fluid loss is maximal, the second phase diuresis could result in a net loss of PV. This postulate needs further investigation; however, there is indisputable evidence that in some cases a microgravity environment, by relieving the stresses of gravity, allows adaptive mechanisms to lapse, resulting in what has been termed cardiovascular deconditioning.

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Table II. Resting cardiovascular response to increased G_z

		+1 G_z	+2 G_z	+3 G_z
\dot{V}_{O_2} , liters \cdot min ⁻¹	a	0.29	0.32	0.35
	b	0.25	0.29	0.40
\dot{Q} , liters \cdot min ⁻¹	a	7.3	5.9	5.6
	b	5.3	4.4	4.3
SV, ml	a	105	66	50
	b	70	47	46
HR, beats \cdot min ⁻¹	a	68	89	115
	b	76	98	115
$\bar{P}a$, mm Hg	a	95	107	115
	b	—	—	—
$(Ca-C\bar{v})_{O_2}$, liters $O_2 \cdot l_b^{-1}$	a	4.1	5.4	8.1
	b	4.8	7.1	8.4

a = Average data from refs 6, 13, 20, 23, and 25; b = average data from our laboratory for the erect posture.

Increased Gravitational Force (+ G_z)

When going from the supine to erect posture, gravity pulls 200–500 ml of blood into the dependent limbs, resulting in a decrease in \dot{Q} and $\bar{P}a$ and an increase in HR. Under normal conditions, the drop in $\bar{P}a$ is not sufficient to lead to orthostatic intolerance. If an individual has a pathological condition or altered cardiovascular reflexes as occurs in the adaptation to microgravity, the effect of gravity may lead to orthostatic intolerance. To this end, studying normal subjects in an increased G_z environment may provide insight into the feedback mechanisms involved in the prevention of or tolerance to orthostatic hypotension. Many studies of G_z tolerance have been conducted; however, relatively few have measured cardiovascular variables during steady state adjustment to increased G_z [6, 13, 20, 23, 25]. Data from these studies are combined with data from our studies in table II. Inasmuch as man can only sustain 3 G_z when unassisted by a G-suit or straining, only data at 1, 2 and 3 G_z are presented.

As indicated in table II, there is an increase in \dot{V}_{O_2} with increasing G-load; this increase is most dramatic at 3 G_z . SV is markedly reduced at 2 and 3 G_z when compared to 1 G_z ; this results in a decreased \dot{Q} in spite of the dramatic increase in HR. The decreased \dot{Q} is offset by a dramatic increase in TPR as $\bar{P}a$ actually increases at 2 and 3 G_z when compared to 1 G_z and the

Table III. Resting cardiovascular responses to prolonged exposure to +G_z

Variable	+G _z	Exposure time				
		4 min	12 min	20 min	28 min	32 min
\dot{V}_{O_2} , liters · min ⁻¹	2	0.24	0.26	0.28	0.29	—
	3	0.41	0.49	0.61	—	—
\dot{Q} , liters · min ⁻¹	2	4.4	4.5	4.4	4.4	—
	3	5.6	5.0	4.6	—	—
SV, ml	2	51	48	44	51	—
	3	47	35	32	—	—
HR, beats · min ⁻¹	2	87	94	99	86	—
	3	120	142	140	—	—
(Ca-C \bar{v}) O ₂ , liters O ₂ · l _b ⁻¹	2	5.4	5.8	5.2	6.6	—
	3	7.3	9.8	13.3	—	—

arterio-venous oxygen difference (Ca-C \bar{v})O₂ is much greater to meet the metabolic demands. At first glance, body position at +G_z did not make a difference; however, the decrease in SV or \dot{Q} and increase in HR are greater in the erect than in the seated position. This difference is presumably due to the greater degree of venous pooling in the erect than in the seated position. The cardiovascular adjustment to 2 and 3 G_z can apparently overcome the increased stress of gravity as the cardiovascular system appears to be in steady state with the only compromise being a reduced cardiovascular reserve. This reduced reserve could become a limitation when the subject is asked to exercise.

In spite of the apparent adjustment to increased G_z discussed above, it is well known that subjects cannot sustain +G_z for very long. We attempted to expose 6 subjects to 32 min resting experiments at 1 and 3 G_z. The data from these experiments are presented in table III. The +2 G_z protocol was tolerated for 20–28 min, while +3 G_z was tolerated for only 12–20 min by which time the subjects developed narrowing peripheral vision. The subjects appeared to maintain their initial adaptations to +G_z. Resting \dot{V}_{O_2} increased with exposure time, especially at 3 G_z, while \dot{Q} was sustained at a level lower than that at 1 G_z until just prior to the decompensation when \dot{Q} decreased (~20%). HR increased as a function of G_z exposure time up to near maximal exposure time when it decreased. Linnarsson [20] has shown that $\bar{P}a$ is increased at +G_z initially; however, as exposure time increases, there is a decrease of $\bar{P}a$ resulting in an inability of the circulatory system to

support the heart-brain pressure gradient, resulting in inadequate brain perfusion. There is a progressive fall in SV and increase in HR during $+G_z$; however, the decrease in \bar{P}_a is much greater than would be expected from the decreased \dot{Q} alone. This suggests that the initial increase in TPR cannot be sustained at either 2 or 3 G_z .

A possible countermeasure to the initial and/or progressive decrease in SV during $+G_z$ exposures could be the muscle-pumping action to increase the VR and therefore SV. In early studies [6, 23], it was suggested that low levels of exercise supported the cardiovascular system during increased G_z ; it was already well known that straining maneuvers assist G_z tolerance [2, 10, 17]. No studies, however, follow the cardiovascular variables over a period of constant G_z . We used low levels of exercise ($\dot{V}_{O_2} = 0.6\text{--}1.0$ liters \cdot min $^{-1}$) during 32 min of 2 and 3 G_z . All 6 subjects completed 32 min at 2 and 3 G_z with a \dot{Q} that was 10–15% above rest and a HR not significantly different from rest at $+G_z$. Both HR and \dot{Q} , and presumably \bar{P}_a , were maintained for the entire 32 min. It would appear that muscle and abdominal/thoracic pumping assisted VR sufficiently to increase \dot{Q} and maintain the increased value for the exposure period.

Adaptation to Work

Although low levels of exercise during $+G_z$ experiments might be useful, their effectiveness during weightlessness remains to be investigated. In addition, the balance between increased VR due to muscle pumping and the increased demand for muscle blood flow and \dot{Q} at increased and decreased G_z should be examined at higher workloads. Data are presented in table IV for the cardiovascular responses to exercise at 0-G (supine), $+1$ and 3 G_z .

Under 1 G_z conditions, \dot{Q} , HR and \bar{P}_a increase linearly with \dot{V}_{O_2} until their maximal values are reached. Under simulated 0 G_z (supine), resting is higher than 1 G_z , HR is lower and \bar{P}_a is similar. As \dot{V}_{O_2} rises, \dot{Q} , HR and \bar{P}_a increase in the 0 G_z condition; however, the differences between 0 and 1 G_z disappear at exercise levels of ~ 2.0 liters $O_2 \cdot$ min $^{-1}$. When compared to 1- G_z values, \dot{Q} is lower at rest and at all levels of exercise at 3 G_z , while HR is significantly greater; \bar{P}_a is also elevated. The delivery of O_2 to tissues is increased in all G_z conditions by increased SV and HR; however, under $+G_z$ conditions, HR dominates while under 0 G_z , SV plays a greater role. The subjects appear to adapt to exercise under all G_z conditions, but the

Table IV. Cardiovascular responses to exercise at 0 G_z , +1 G_z and +3 G_z for seated exercise from the literature (a) and for our erect exercise data (b)

Variable	\dot{V}_{O_2} , liters \cdot min $^{-1}$			
	G_z	rest	1.0	2.0
\dot{Q} , liters \cdot min $^{-1}$	0	7.3	12	15
	1	6.3	11	16
	3a	5.6	10	—
	3b	4.8	8	13
HR, beats \cdot min $^{-1}$	0	68	90	137
	1	72	99	134
	3a	115	150	—
	3b	115	148	172
\bar{P}_a , mm Hg	0	94	106	122
	1	95	105	125
	3a	115	125	—
	3b	—	—	—

mechanisms of the adjustments are different. For example, at a \dot{V}_{O_2} of 1.0 liters \cdot min $^{-1}$, the $(Ca-C\bar{v})_{O_2}$ is 8, 9.1, and 12 liters O_2 /1 blood for 0, 1, and 3 G_z , respectively. At the higher workload $(Ca-C\bar{v})_{O_2}$ was 13, 13, and 15 for 0, 1 and 3 G_z , respectively. As higher workloads are achieved, the increased VR due to the supine posture is no longer evident, while on the other hand, the decreased VR due to + G_z becomes a major limitation to \dot{Q} . The limit to the adjustment of the cardiovascular system can be considered to be the maximal aerobic power. Previous work has shown that the \dot{V}_{O_2} max in the supine position is 10–15% lower than in the erect position in spite of the greater SV at lower workloads [5, 24, 28].

On the other hand, it is obvious from table IV that the maximal \dot{V}_{O_2} at 3 G_z is significantly lower than at 1 G_z ($\sim 40\%$). In spite of the limitations of increased G_z on the cardiovascular system, exercise can be carried out at least at modest workloads while the response of the cardiovascular system at 0 G_z is not appreciably different than at 1 G_z . It should be noted, however, that at low exercise levels ($\dot{V}_{O_2} < 1.0$ liters \cdot min $^{-1}$) 3 G_z can be tolerated for 30–45 min while at higher workloads ($\dot{V}_{O_2} > 1.0$ liters \cdot min $^{-1}$), G_z tolerance time is reduced to 16–24 min. This would suggest that the cardiovascular system is not in a true steady state. Further investigation into this area is needed.

Adaptation to the space environment requires first the adjustment to weightlessness and then re-adaptation to gravity. Astronauts will be required to perform work in both a weightless environment and under increased gravitational force. The ability to adapt to exercise over time is critical to successful adaptation. Exercise during weightlessness is apparently not limited at submaximal levels. As discussed above, under increased G_z conditions, there is an initial adjustment of the cardiovascular system; however, in a relatively short period of time, the cardiovascular system cannot meet the demands of G_z plus exercise. It is not apparent that these observations could be applied to a 1 G_z environment following adaptation to weightlessness; however, the response to 1 G_z after adaptation to weightlessness seen as cardiovascular deconditioning would appear similar to that made in going from 0 to 1 G_z . In studies by Convertino [8, 9], exercise performance after 10 days of HDT or bed rest (0 G_z) demonstrated that \dot{V}_{O_2} max was lower ($\sim 8\%$), submaximal HR was greater ($\sim 5\%$), and the anaerobic threshold lower. This author concluded that PV lost during adaptation to weightlessness resulted in the observed cardiovascular deconditioning. Interestingly, Convertino's findings are qualitatively similar to the data where 2 G_z is compared to 1 G_z .

In summary, it appears that man is capable of adapting to a weightless environment. Although the application of the Gauer-Henry hypothesis to this adjustment needs to be reconsidered, there appears to be a decrease in PV as well as in sympathetic tone after 2–3 days of 0 G_z . The role of the hydration state of the subjects needs further investigation, as there appears to be complete adjustment to 0 G_z within 8 h without a decrease in PV (when subjects are allowed to dehydrate mildly). In actual space flight, space motion sickness is experienced at least mildly by most astronauts. This may alter the hydration state for the first few days, and once relieved by rehydration, may lead to the decrease in PV that is typically reported. Hydration changes account for the inability to re-adapt to gravity upon return to earth and may lead to the inability to function when landing on other planets. Apparently exercise, per se, does not serve as an effective countermeasure. Even though $+G_z$ tolerance was improved, it did not persist long enough to maintain the steady state.

Given sufficient adjustment time, man can re-adapt to gravity; however, the dynamics of the adjustments of the cardiovascular system need further investigation. It is clear that, if man is to successfully adapt to the space environment and return from it, we must prevent cardiovascular deconditioning that develops during the weightless period by either uncov-

ering and interfering with the mechanism that causes it or by developing effective countermeasures. These could presumably be used not only during weightlessness but also during re-entry. By identifying and using such countermeasures, we may be able to effectively extend man's interplanetary initiatives as he learns to cope with gravitational force in much the same way as he deals with other environmental variables.

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SKELETAL MUSCLE

RESEARCH ON THE ADAPTATION OF SKELETAL MUSCLE TO HYPOGRAVITY: PAST AND FUTURE DIRECTIONS

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ABSTRACT

Our current understanding of hypogravity-induced atrophy of skeletal muscles is based primarily on studies comparing pre- and post-flight properties of muscles. Interpretations are necessarily qualified by the assumption that the stress of reentry and readjustment to terrestrial gravity do not alter the parameters being analyzed. The neuromuscular system is highly responsive to changes in functional demands and capable of rapid adaptation, making this assumption questionable. A reexamination of the changes in the connective tissue and synaptic terminals of soleus muscles from rats orbited in biosatellites and sampled postflight indicates that these structural alterations represent adaptative responses of the atrophic muscles to the increased workload of returning to 1 G, rather than hypogravity per se. The atrophy of weightlessness is postulated to result because muscles are both under-loaded and used less often. Proper testing of this hypothesis requires quantitation of muscle function by monitoring electromyography, force output and length changes during the flight. Experiments conducted in space laboratories, like those being developed for the Space Shuttle, will avoid the complications of reentry before tissue sampling and allow time course studies of the rate of development of adaptive changes to zero gravity. Another area of great importance for future studies of muscle atrophy is inflight measurement of plasma levels of hormones and tissue receptor levels. Glucocorticoids, thyroid hormone and insulin exert dramatic regulatory influences on muscle structure. Prevention of neuromuscular atrophy becomes increasingly more important as spaceflights increase in duration. Definition of the atrophic mechanism is essential to developing means of preventing neuromuscular atrophy.

INTRODUCTION

Skeletal muscle atrophy, especially of the antigravity muscles, continues to represent a major problem to long term habitation in the hypogravity environment of space by man. Research in this area has been directed toward defining the atrophic process and finding means of preventing it. The primary issues, as yet unanswered, are to define what attributes of weightlessness are deleterious and to determine whether the atrophic process is similar to that occurring on Earth during periods of inactivity such as extended bed rest. Does the hypogravity-induced atrophy simply represent quantitative and qualitative changes in the components of the cells of the neuromuscular system? From information available to date it is not possible to rule out that a more serious situation exists in that there is cell death as in denervation atrophy. If the latter is true, then unique means of preventing and reversing muscle wasting will have to be developed.

Our current understanding of hypogravity-induced atrophy is based largely on the results of animal experiments flown in the Cosmos series of biosatellites and human studies during the Gemini, Skylab, Apollo and Soyus missions [1]. As recognized by previous investigators, characterization of the effects of hypogravity on the neuromuscular and other systems has been handicapped by the return of the biosatellites to 1 G and the lapse of 4.5 hrs to 2 days prior to tissue sampling. Interpretations must be qualified by the assumption that the parameters being analyzed are not altered during this period. Unfortunately, dramatic changes can, and probably do, occur in the neuromuscular system, rendering this assumption highly questionable. In the present discussion, selected issues will be reassessed from past biosatellite data to form a framework upon which the need for specific future directions of research will be discussed.

POSTFLIGHT TISSUE SAMPLING OF RATS FLOWN IN BIOSATELLITES

Muscle Fiber Atrophy and Connective Tissue Proliferation: The soleus muscles of rats flown

22 days aboard Cosmos 605 were examined morphologically on the second day postflight [2]. Muscle wet weight had decreased significantly by 32%. Marked focal edema of the endo- and perimysial layers and increases in connective tissue cells were found as well as focal disruption of muscle fibers. It was theorized that these changes were due to the hypokinesia of weightlessness which produced blood stasis, edema and connective tissue proliferation that interfered with the vascular trophism of muscle fibers and caused their partial disintegration. Similar changes were reported for an Earth-based model of hypokinesia which involved rigidly-restraining rats from moving. Following a few days of restraint, the edematous appearance and focal increase in connective tissue cells was evident in the soleus muscles [3]. Unlike the muscles from rats flown on Cosmos 605, muscle fiber atrophy was not present. In this case, the hemodynamic disorder was postulated to have developed from a combination of mechanical compression of the hindlimb vessels by the restraint mechanism, and from inadequate venous return from the quiescent muscle which was not exerting its normal pumping action to aid venous return. Analysis of soleus muscles 4.5 to 5 hrs postflight, as occurred for Cosmos 782 and 936, revealed that the edema was absent and therefore, it must be a postflight phenomenon occurring in the first 2 days [4]. It was concluded that the deconditioning of the muscular and vascular systems during weightlessness rendered the muscle incapable of handling the increased blood flow which accompanied resumed movements and therefore, stasis, followed by edema, resulted. No direct measurements of blood flow were made in this system. More recently, blood flow was demonstrated to be reduced during drug-induced flaccid paralysis and may have played a role in atrophy [5]. However, we would like to propose an alternative explanation for the biosatellite data:

Jablecki *et al.* induced rapid hypertrophy of normal soleus muscles in rats by tenotomizing synergistic muscles, i.e., the distal tendons of the plantaris and gastrocnemius muscles were severed and the soleus tendon spared, resulting in the soleus muscle acquiring the additional workloads of the tenotomized muscles [6]. After 2 days of contracting against the increased workload, the solei exhibited widened intercellular areas with a proliferation of fibroblasts and macrophages. The fibroblasts were heavily labeled following injection of a radiolabeled RNA precursor, uridine, and there was a definite increase in connective tissue matrix seen ultrastructurally. Proliferation of the connective tissue was found biochemically for hypertrophying muscles in a similar experiment [7]. The transition of the atrophic muscles from the hypogravity environment to terrestrial gravity constituted a dramatically increased workload and stimulated rapid growth. In the restricted movement model, the rat would be expected initially to struggle strenuously to free himself. The soleus muscles would be contracting isometrically while being held in a lengthened state which together would promote hypertrophy [8]. The so-called "edema" appeared to be the growth response of the connective tissue to the increased force rather than venous congestion. Although vascular congestion cannot yet be ruled out for the rapid hypertrophy experiment, the vascular and muscular systems of the soleus muscles at the onset of the increased workload were normal, unlike those of the deconditioned muscles of flight animals, and presumably were capable of handling the increased blood flow.

Degeneration of Neuromuscular Junctions: The motor innervation of skeletal muscles of adult rats consists of myelinated preterminal axons which synapse at the endplate as a fine terminal arborization (Figure 1). At the ultrastructural level, spherical synaptic terminals filled with "50 nM" vesicles and mitochondria lie within synaptic clefts formed by the plasmolemma of the muscle fiber (Figure 2).

Soleus muscles from Biosputnik 936 were processed for ultrastructural analyses 4.5 to 9 hrs postflight [9]. Synaptic terminals in the muscles from vivarium control animals exhibited high synaptic vesicle density and intact mitochondria similar to that in Figure 2. Terminals in the muscles from rats flown in space had significantly fewer synaptic vesicles and the mitochondria were markedly swollen. These changes were thought to represent the initial stages of degeneration of terminals resulting from hypokinesia. On the contrary, a more plausible explanation is that the changes were the result of hyperactivity of the neuromuscular junctions following the return to 1 G. We conclude this because electrical stimulation of the nerves of normal muscles produces comparable decreases in synaptic vesicle number and mitochondrial properties within a few hours [10,11]. It is evident from this and preceding examples that inflight tissue sampling, uncomplicated by the stresses of reentry and early adaptive responses to 1 G, is essential for characterizing muscle atrophy. This will be possible in the Space Laboratory carried into orbit by the Space Transportation System.

RESPONSES OF MOTOR INNERVATION TO ALTERED ACTIVITY

Necessity of Inflight Studies of Motor Innervation: There are sufficient indications from Earth based and biosatellite studies that the innervation of skeletal muscle is disrupted during both disuse and hyperactivity to warrant concern about the integrity of this system. Tenotomy reportedly resulted in decreases of average motor endplate area, the size of the terminal arbor, the diameter of the myelinated axons and disturbingly, the total number of

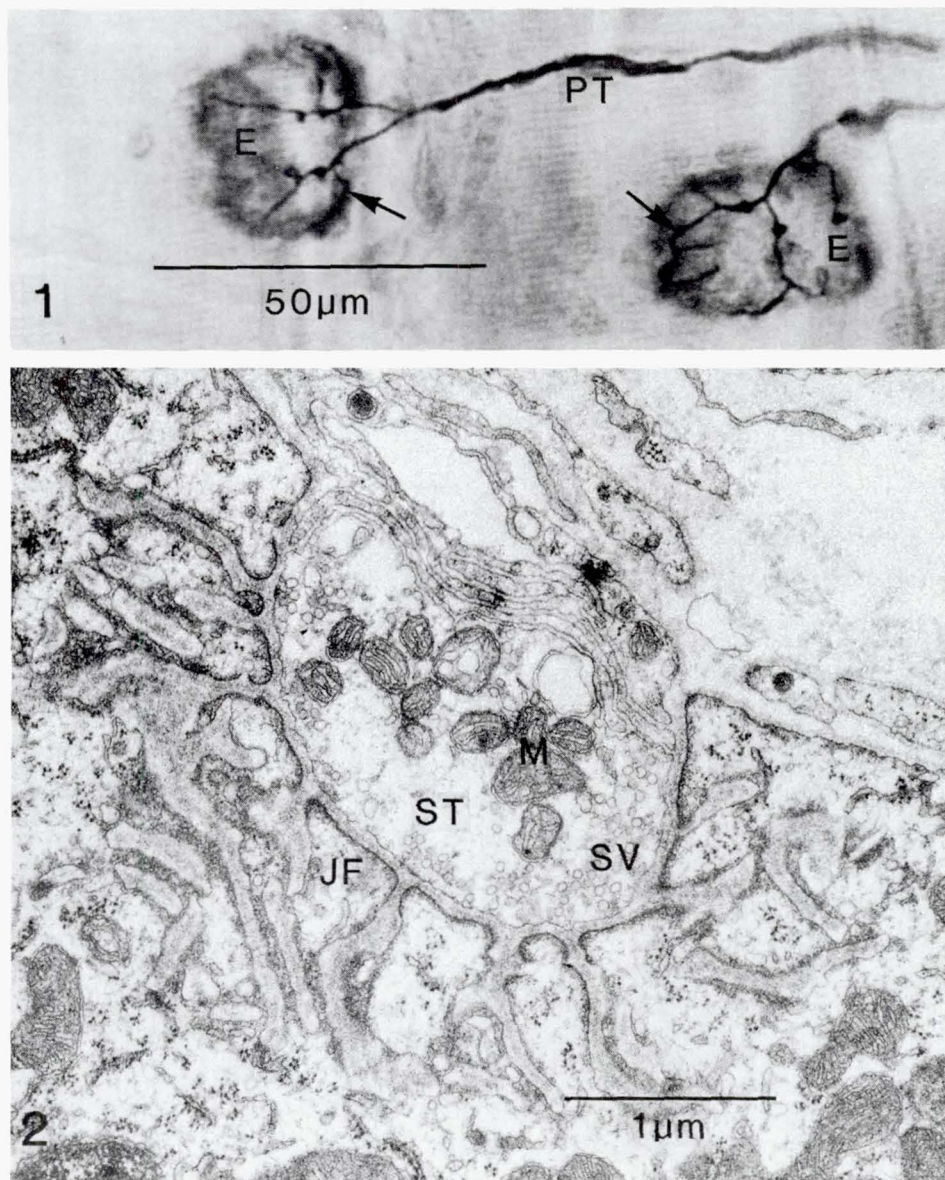


Figure 1. Two neuromuscular junctions of a normal rat skeletal muscle demonstrated by a combination of silver impregnation of the motor axons and histochemical staining of the endplate acetylcholinesterase. The cross-striations of the underlying muscle fibers are visible. The axons consist of preterminal (PT) myelinated portions and nonmyelinated terminal branches (arrows) ramifying over the cholinesterase reaction product (E). At the ultrastructural level, a cross section of a terminal branch would appear similar to Figure 2. Bar equals 50 μ m.

Figure 2. A portion of a rat neuromuscular junction in a normal soleus muscle. The synaptic terminal (ST) lies within a depression, the synaptic cleft, and apposes the postsynaptic membrane of the muscle fiber which is thrown into numerous junctional folds (JF). Within the terminal, "50 nm" clear synaptic vesicles (SV) are clustered near the sides of the terminal adjacent to the postsynaptic membrane. Mitochondria (M) are aggregated centrally. Bar equals 1 μ m.

axons in the muscle nerve [12,13]. Immobilization resulted in a significant decrease in average axon diameter, but the total number of myelinated axons was unchanged [14]. Interestingly, the axons increased in diameter in the nerves of the muscles contralateral to the intact side. Chronic exercise training increased axon diameters in the mouse [15]. Rapid hypertrophy of the rat soleus muscle following inactivation of synergistic muscles and strenuous exercise produced enlargement of the motor endplates and preterminal motor

axons [16]. Following short bouts of intense exercise, swelling of motoneurons and increased acid phosphatase activity were found [17,18]. Acid phosphatase activity increases when axons were injured [19]. In the restricted mobility experiments, destruction of end-plates, sprouting of motor axons and loss of muscle fibers were detected in the rat soleus muscle [20]. Thickening and coarsening of nerve terminals was reported for rats orbited in the Cosmos biosatellites [21]. It cannot be determined without further experimentation whether these changes in the flight muscles resulted during reentry to Earth's gravity or the exposure to hypogravity. Inflight sequential biopsies of the neuromuscular system are required to resolve this issue.

Masking of Motor Innervation Deficits by Compensatory Mechanisms: Large losses of motor axons could occur without an obvious long term deficit because partial denervation of a muscle induces the remaining healthy axons to sprout and reinnervate the orphaned muscle fibers [22]. Function is returned relatively quickly because of the short distance for axon growth. Muscle tension would fall initially following axon loss, but it would return to normal as reinnervation and compensatory hypertrophy of the innervated muscle fibers proceeds. The compensatory process could conceivably mask a motor nerve deficit after an initial jaunt into space. Even though the system repairs itself, this does not mean that there is no reason for concern because the capacity of axons to sprout is limited [22]. Repeated exposures to weightlessness could eventually exhaust this mechanism and the neuromuscular deficit would become more pronounced and eventually, debilitating. The integrity of the motor innervation should be assessed by light and electron microscopic analyses of inflight tissue biopsies.

NEEDS FOR QUANTITATION OF MUSCLE FUNCTION

Definition of Muscle Function: Muscle atrophy of hypogravity is postulated to occur because the muscles are both underloaded and used less often. To test this hypothesis in animal experiments requires simultaneous measuring the pattern of muscle activity (electromyography), force output and muscle length. These data are obtained by direct wiring or biotelemetry of electromyography (EMG) signals from implanted intramuscular electrodes, force transducers attached to the muscle tendon and a length gauge anchored in parallel with the muscle of interest [23,24]. The EMG signals provide information about the duration of contraction and average frequency of firing. Additional information is extractable from the EMG signal by frequency spectral analysis which treats the muscle action potentials as a summation of simpler waveforms and differentiates the complex EMG signals into basic components occurring in preselected frequency bands [25]. The power (amplitude) of each of these frequency components is compared. The dominant or mode frequency of a spectral histogram shifts significantly in myopathies and neuropathies [26]. These changes correlate with variations in the shape and duration of the normal bi- and triphasic muscle action potentials to more polyphasic potentials. This approach proved a sensitive measure of muscle dysfunction in astronauts having experienced as little as 9 days of hypogravity in the Apollo-Soyuz space mission [27,28]. Postflight fatiguability increased significantly, and this was indicated by spectral power shifts into lower frequencies during exercise testing. Muscle atrophy was indicated by an increase of the mode frequency from the preflight baseline 55 Hz band to the 95 Hz band. Spectral analysis performed pre-, in- and postflight on astronauts would allow definition of the onset and degree of atrophic changes and provide valuable feedback on the effectiveness of inflight prophylactic measures and postflight recovery procedures.

Length is an important parameter because the strength of a muscle contraction is dependent on the degree of overlap of the actin and myosin [29]. A highly active muscle, assessed electromyographically, can generate a small amount of tension if it is either extended or shortened greatly beyond its normal working midrange. Measurements of length and EMG activity in unrestrained cats proved very effective in defining the differential participation of soleus and medial gastrocnemius muscles in standing, walking, running and jumping activities [24,30]. The soleus muscle was very active in standing whereas the medial gastrocnemius was nearly silent. As the intensity of the movement increased from walking to running the medial gastrocnemius became more active and generated maximum force only during jumping. Pre-, in- and postflight monitoring of these parameters in instrumented animals would provide valuable data on altered muscle function in weightlessness, the efficacy of prophylactic measures, the recovery process, and for comparison with biochemical and anatomical changes in the muscles.

Advantage of Quantitating Muscle Function: A survey of studies examining muscle adaptation to hypogravity and terrestrial model systems reveals a serious lack of quantitation of muscle function. Without this information, there is no precise definition of the conditions to which the muscle is adapting. For example, consider the following situation in which the application of electromyography to assess muscle activity greatly advanced the understanding of muscle atrophy produced by tenotomy. Cutting one tendon of a muscle and allowing the muscle to hypershorten results in severe atrophy. Previous investigators hypothesized that the atrophy was the consequence of the combination of removing the

weight bearing function of the tenotomized muscle and decreasing its stretch-activated contractile activity. While atrophy occurred in tenotomized soleus muscles of the cat, rat and rabbit, daily EMG recording revealed, unexpectedly, that the normal pattern of nearly continuous activity was dramatically decreased in all solei, except that of the cat in which contractile activity remained at the control level [31,32,33]. In fact, totally eliminating activity of tenotomized muscles by spinal cord transection, which removed supraspinal activation of soleus motoneurons, markedly retarded muscle atrophy. Conversely, hyperactivity, achieved by electrical stimulation of the nerve supplying the tenotomized muscle, significantly accelerated muscle degeneration [34]. The changes in muscle use following tenotomy were not uniform in different species which indicated that neither workload nor activity *per se* were the primary causes of atrophy. The common factor was recognized to be hypershortening of active muscle fibers which resulted in decreased fiber diameter and disruption of their myofibrils. This conclusion would not have been reached without the knowledge of activity patterns.

Motor Unit Function: Large electrodes are used to monitor gross muscle activity and smaller electrodes are used to assess function of single motor units i.e., all of the muscle fibers innervated by a single α motoneuron [35,36]. Electrode configuration and placement are important because all human skeletal muscles are composed of a mixture of fast and slow motor units which differ in their normal patterns of activity. Fast units are active in brief bursts occurring infrequently whereas slow units exhibit frequent bouts of nearly continuous activity. Chronic recording of EMG in a variety of human muscles demonstrated that muscles with the highest proportions of slow fibers were active more often than those containing a high percentage of fast units [37]. Patterns of EMG activity were shown for the rat diaphragm and to correlate with the fiber type population in the field of the recording electrodes [38]. The rat soleus muscle, an antigravity muscle, exhibits continuous low frequency (10 Hz) activity when the rat is standing in 1 G. Immobilization of the hindlimb shifted muscle activity to a phasic pattern, i.e., a pattern of infrequent, brief trains of 10 Hz contraction [36]. One would predict a similar shift in hypogravity. Investigation of this hypothesis requires quantitation of muscle function from humans and animals in space.

NEED FOR INFLIGHT MONITORING OF HORMONES

Glucocorticoids: Inactivity of weight bearing muscles due to immobilization appears to increase the number of glucocorticoid receptors in the muscle, and presumably results in an increased sensitivity to circulating glucocorticoids [39,40,41]. Reduction of muscle mass and an impairment of performance may then be expected to ensue in the immobilized or disused muscles. It remains to be determined whether muscles subjected to disuse from weightlessness also increase their receptor number in parallel with the selectivity and degree of atrophy that are especially noticeable in postural muscles e.g., the soleus muscle.

The consequence of increased numbers of glucocorticoid receptors in disused muscles may be augmented by increases in the concentrations of plasma glucocorticoids. Although inflight measurements of plasma corticosteroids are not available, the adrenal hypertrophy observed in rats flown in biosatellites suggests that a chronic stress response occurs on exposure to weightlessness for 20 days [39,40] in which case plasma corticosteroids should be elevated. Moreover, significant increases in corticosteroid dependent enzymes (glutamic-oxalacetic transaminase and glutamic-pyruvic transaminase) in the livers of rats also indicate that plasma corticosteroid levels were elevated during spaceflight [39].

Ground-based research and inflight muscle sampling in test animals is strongly indicated with respect to analyses of glucocorticoid receptors as well as the concentrations of glucocorticoid and other hormones in plasma such as reported for man in spaceflight by Leach *et al.* [39].

Insulin: An interesting effect of immobilization on skeletal muscle, and one which we might expect to find produced also by weightlessness, was observed with regard to insulin sensitivity. The *in vitro* uptake of 2-deoxyglucose and glycogen synthesis by soleus muscles excised from the immobilized hindlimb of mice was significantly decreased in response to insulin as early as 24 hrs after the beginning of immobilization [42]. The direct demonstration of reduced insulin sensitivity of inactive skeletal muscles of mice is in keeping with the commonly observed reduction in insulin sensitivity of man during conditions of inactivity induced by extended bedrest [43]. Whether there are relationships between postural activity, muscle fiber types, susceptibility to atrophy, and insulin sensitivity of specific muscles in the limbs of experimental animals are subject for future investigation.

Thyroid Hormone: The potential importance of plasma hormone concentrations in regulating muscle characteristics and, consequently, performance is illustrated in the case of thyroid hormone. We have recently found that lowering thyroid hormone levels by thyroidectomy dramatically shifts the myofibrillar ATPase activity of the fast fibers in the rat soleus muscle from high to low activity with no apparent gross change in the use of the hindlimb

[44]. That the decreased level of thyroid hormone was working through an intact innervation was indicated by the lack of a similar response in denervated muscles of hypothyroid rats. Thus, in future studies of flight animals and man blood analyses of relevant hormones should be monitored inflight to ascertain their possible contribution to muscle atrophy.

FUTURE DIRECTIONS OF NEUROMUSCULAR RESEARCH IN SPACE

For the evaluation of the effects of weightlessness on skeletal musculature, one of the primary requirements is tissue sampling during the course of flight. This capability is desirable for two reasons: One is to avoid the complicating effects of reentry acceleration forces as well as the subsequent exposure to the Earth's gravity before muscle sampling or sacrifice of the experimental animal. The second consideration is that an inflight sampling capability would allow time course studies of the rate of development of adaptational changes to zero gravity.

In the case of human subjects, there is good evidence that hypogravity induces the loss of muscle mass and strength in the lower limbs [45]. However, it is not yet known which lower limb muscles are effected most severely and to what degree, particularly with regard to rate of atrophy inflight as well as recovery postflight. Computer assisted tomography of the lower limbs, performed pre- and postflight, may provide quantitative evidence of the reduction in size of specific muscles as well as data concerning their water, fat, and protein content [46,47]. Information regarding the status of individual muscles would be useful in the design of prophylactic exercises.

The metabolic status of skeletal muscle with regard to high energy phosphate compounds is likely to be altered during hypogravity-induced atrophy. Creatine phosphate, ATP, and sugar phosphates may be determined by phosphorus nuclear magnetic resonance (NMR) measurements on excised muscles obtained during the course of the mission from experimental animals and subsequently analyzed in ground based NMR facilities [48,49].

Advancing our understanding of muscle adaptation requires that a substantial emphasis be placed on quantitation of muscle function by measuring the pattern of muscle contraction and tension generation in response to varying workloads. The major theories of hypogravity-induced atrophy, hypodynamia and hypokinesia, can be evaluated with this information obtained from pre-, in- and postflight muscles. In a reverse sense, the validity of using model systems, such as restricted mobility [3] and suspended restraint [50,51], for simulating weightlessness is testable by comparing muscle function in these systems with that obtained in flight. Having suitable models for study could reduce the number of costly flight experiments. Furthermore, the development of prophylactic measures of preventing atrophy is aided by objective measures of the success of the procedures, i.e., determination of the minimum amounts of exercise and artificial gravity required to avert muscle wasting during space flight [45,52].

As man enters the unknown environment of space for longer periods, the continuation of research on his adaptation becomes more crucial. The biosatellite studies of the Russian scientists made important pioneering contributions to this field for which they should be congratulated. The continued cooperation and exchange of information between space scientists of the world should continue because these efforts benefit all peoples.

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MACULAR BIOACCELEROMETERS ON EARTH AND IN SPACE

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Abstract

Spaceflight offers the unique opportunity to study linear bioaccelerometers (vestibular maculas) in the virtual absence of a primary stimulus, gravitational acceleration. Macular research in space is particularly important to NASA because the bioaccelerometers are proving to be weighted neural networks in which information is distributed for parallel processing. Neural networks are plastic and highly adaptive to new environments. Combined morphological-physiological studies of maculas fixed in space and following flight should reveal macular adaptive responses to microgravity, and their time-course. Ground-based research, already begun, using computer-assisted, 3-dimensional reconstructions of macular terminal fields will lead to development of computer models of functioning maculas. This research should continue in conjunction with physiological studies, including work with multichannel electrodes. The results of such a combined effort could usher in a new era in understanding vestibular function on earth and in space. They can also provide a rational basis for counter-measures to space motion sickness, which may prove troublesome as space voyagers encounter new gravitational fields on planets, or must re-adapt to 1 g upon return to earth.

Introduction

Before humans actually flew in space, there was great fear that space would prove too hostile an environment to withstand. Among the predicted effects of weightlessness were those related to the balance end organs of the inner ear, which are finely tuned to earth's gravitational field. Some medical scientists believed that humans would become severely disoriented and unable to function in space (see Deitlein, 1977). The vestibular system was, therefore, looked upon as a prime candidate for study in space and high priority was given

Keywords: macula, 3-D reconstructions, neural network, space adaptation

to it. These fears, however, did not materialize and interest waned. During the first suborbital flights, when astronauts were closely confined within the vehicle, there were no vestibular-related problems at all. It was only later when spacecraft became larger, permitting movement (particularly of the head), that the syndrome called space motion sickness, or space-adaptation syndrome, appeared. Even then, statistics compiled have shown that only about half the astronauts and cosmonauts become ill, and that the syndrome tends to disappear during the first 2-4 days of flight (Nicogossian and Parker, 1982). It is unclear in the remaining cases whether prophylaxis accounts for some of the resistance to the syndrome, or if some individuals are simply immune. A further interesting fact is that there does not appear to be a relationship between motion sickness in earth's gravitational field and proneness to space motion sickness. Thus, there is currently no means of assessing an individual's inclination to become sick in space through ground-based testing. Upon return to earth, there is a re-adaptation to the 1 g field which varies somewhat in symptoms and in severity from one individual to another (Homick and Miller, 1975; Homick *et al.*, 1977). The period of re-adaptation is roughly proportional to that of the flight (Kornilova *et al.*, 1979) as is also known to occur in sea-sickness. The finding that symptoms of re-adaptation are more universal in astronauts and cosmonauts than are those of space sickness would suggest that all individuals experience an adaptation to weightlessness, whether or not frank illness is a complement of the process.

This paper will consider recent findings which indicate that the vestibular system is most worthy of regaining its place of importance in the NASA space program. The system is highly significant because of its functional organization as a parallel processing, weighted neural network. Such networks are considered to be plastic and highly adaptive to new environmental factors. Maculas, the linear bioaccelerometers of the vestibular system, should, therefore, undergo adaptive changes in response to microgravity. Once adapted, they must re-adapt to earth's gravity, or adapt again to a different, partial gravitational field if that is encountered. This has long-term implications as we look forward to permanent stations on the moon, or to exploration of Mars. Of equal importance is the fact that macular tuning to various gravitational environments can serve as a relatively simple model in the study of central neural adaptation, which must occur as the brain resolves conflicts between visual, kinesthetic and vestibular information in space. Finally, maculas are amenable to computer modelling to predict, simulate, and test adaptive processes.

The Peripheral Receptor as a Parallel Processor

Recent research with long series of sections through rat maculas has shown that type II cells lack an independent innervation to the central nervous system. They synapse with calyces of type I hair cells and with collaterals of calyces (Ross, 1985, 1986). Such synapses were observed first by Ades and Engstrom (1965), although the universality of the arrangement was not appreciated at the time. Much earlier, Lorente de Nó (1926) had described calyceal collaterals in his Golgi studies of mouse maculas, although the existence of two kinds of hair cells was not understood.

The new results shed a different light on the functional organization of the macular receptors. (It should be noted that ampullary end organs appear to have the same kind of

organization, although we have not yet studied them systematically.) Maculas have the functional organization of a data-flow computer that is suitable for parallel processing of information in real-time. What is the basis for this conclusion, and what are the questions raised?

Morphological Findings

Types of Nerve/Terminal Innervation Patterns

Work with montages showed that there are three major kinds of nerve/terminal fields in rat maculas, M, M/U and U, based upon the presence (or absence), and the length, of the unmyelinated pre-terminal segment. These are M-, U- and M/U-types (Ross, 1986).

The M-type essentially lacks a pre-terminal segment, because the nerve is myelinated to the calyx. The nerve does not penetrate the neuroepithelium, but the basal cells and basal lamina closely surround it as it rises to the calyx, which does lie within the macula proper. The myelin of these large-diameter nerves unwraps in an unusual manner at the initial node of Ranvier, situated at the calyx. Some of the leaflets end slightly in advance of the others, so that the portion closest to the calyx has fewer layers of myelin and is also enlarged. One calyx comprises the terminal, which usually contains several type I cells and lacks collaterals.

The U-type is the most complex. The thinly myelinated, smaller diameter nerve loses its myelin below the neuroepithelium and becomes surrounded by processes of the basal cells. The unmyelinated, pre-terminal segment enters the neuroepithelium distal to its actual termination and sometimes hooks around other unmyelinated nerves in its course. It may give rise to a single calyx with subparts, in which case the calyx splits above the cell bodies to follow closely the necks of the enclosed type I cells as they reach towards the heads at the macular surface. In other cases, common in the area of the utricle most completely investigated here, the nerve bifurcates into branches of unequal length. Each branch gives rise to a single calyx containing a single type I cell. Other variations occur, with combinations of calyces with multiple and with single cells not uncommon. In general, calyces of the U-type terminal field have several collaterals, some of which have efferent morphology. Afferent collaterals supply type II cells related to the terminal's field of innervation, and also type II cells more closely related to other terminal fields. Efferent-type collaterals tend to extend to type II cells of the terminal's own field. Particularly in the case of afferent-type collaterals, reciprocal or sequential synapses are common. That is, both a ribbon or spherule junction and a subsynaptic cistern are present in the type II hair cell, and vesicles accumulate near the cisterns on the presynaptic side.

The M/U-type is intermediate with respect to nerve diameter and terminal complexity. Branching of the pre-terminal segment often occurs but collaterals are infrequent. Only those nerves which have short, unmyelinated, pre-terminal segments are categorized as M/U-type.

A different terminology introduced by Goldberg and his colleagues (Goldberg *et al.*, 1985) is based upon the configuration of the calyx alone. That is, whether the terminal has both calyceal and bouton endings (dimorphic), has only a calyceal terminal (calyx), or only bouton endings (bouton units). A further part of this classification is that simple calyces supply individual hair cells and complex calyces supply 2-3 adjacent hair cells.

It will be important in the near future to agree upon one standard terminology that is

meaningful both physiologically and morphologically. In the context of neural networks, we have found the classification by type of nerve/terminal innervation pattern to serve best for development of concepts. The terms M-, U-, and M/U-types bring to mind a particular set of characteristics (outlined above) useful in conceptualizing corresponding physiology. Our morphological findings are that the length of the pre-terminal segment is related to the size of the nerve fiber and its myelination, to opportunity for anatomically close apposition of unmyelinated segments, and to the complexity of the calyceal terminal. (By complexity, we mean the presence of numerous collaterals.) Physiologically, differences in length of a pre-terminal segment imply differences in timing of initiation of response, and size and degree of myelination are reflected in conduction speed of the parent nerve. This is of significance in dealing with the neural networks they form, because network calyces of all 3 kinds of nerve/terminal patterns are addressed by the same type II hair cell in some portions. This would imply chronological encoding of information passed to the central nervous system (see below). Encoding by nerve response characteristics may be reflected in the discreteness or complexity of the terminal field, and in opportunity for electrical coupling of unmyelinated pre-terminals which have long intramacular courses (discussed below).

Computer-assisted 3-D Reconstruction of Macular Nerve/Terminal Fields

Visualization of the hair cells, calyces, and nerves of the maculas has been greatly enhanced by computer-assisted reconstruction of slices through the structures, traced from electron micrographs. The first reconstruction attempted was that of an M-type field, because of its relative simplicity. All of the type II cells synapsing with this type of terminal do so upon the calyx, as no collaterals are present. This simple example required 370 sections to include the calyx and all cells (5 type I, 7 type II) which synapsed with it. The program employed (Kilgore *et al.*, 1984) was capable of illustrating a calyx, type I cells and type II cells individually or in small groupings, but could not show this most simple terminal field in its entirety. Neither was it possible to illustrate kinocilia or stereocilia. The program, while an important first step, simply was not sophisticated enough to handle all the data points required.

Nevertheless, the resulting reconstructions were useful in many ways. They showed, for example, that the type I cells within the calyx did not have identical orientations at their heads. Even in the absence of visualization of the kinocilia and stereociliary tuft, this meant that these cells, at least, did not have identical polarizations. The evidence about type II cell orientations was less clear, because of the inability to construct the entire field with the aid of a computer. The available data did, however, strongly suggest that no two hair cells had identical polarization. A further observation made during the process of reconstruction was that type II cells aggregated into clusters at non-periodic intervals. The clusters usually consisted of 4-7 cells in the region studied, but sometimes only two cells were involved. The cells had closely interdigitating borders, but no synapses were observed.

A further benefit of the reconstructions was the ability to estimate the portion of the macular surface covered by cells innervated by the terminal. For the type I cells, this area is irregular in outline and is ~138 μm long x 25 μm wide. One of the heads of a type II cell lay outside this area, extending the width another ~7 μm at this site. This portion of the macular surface, then, corresponds to the sensory field of the terminal. Its dimensions are very

different from those of the calyx itself which, in this example, is the terminal field since there are no collaterals.

A more complete reconstruction of this terminal and its hair cells has now been accomplished (Fig. 1) with the aid of a different program (Kinnamon, 1986). All of the cells as well as the calyx are visualized in one image, and the kinocilia can be projected in place. This reconstruction verifies the locations and angles of the heads at the macular surface. The angles of polarization of the cells are, however, still too difficult to determine with any reasonable degree of accuracy. Future programs must be devised to slice through the basal bodies of the kinocilia of the hair cells, to resolve this problem.



Fig. 1. This reconstruction illustrates an entire M-type terminal field with 5 type I cells (blue), 7 adjacent type II cells (red), the calyx (green) and kinocilia (white).

Use of the Kinnamon program has permitted us to image examples of all three kinds of nerve/terminal innervation patterns, and to illustrate linkages between terminals. An example of a U-type pre-terminal with a broad calyceal collateral (afferent-type) to a type II hair cell is shown in Figure 2. This interesting pre-terminal split into two branches of unequal length. Each branch ended in a single calyx which held but a single type I cell. Each calyx had 3 type II cells synapsing with it, so that the terminal innervated a total of eight hair cells.

It should be noted that the bifurcating type of pre-terminal was the most common kind observed in network 2 (Fig. 4). Most frequently, each calyx has its own complement of type II cells which do not communicate with the other calyx of the pair, but which do synapse on other, nearby terminals. It is as though each branch has its own sensory field. Sometimes these are close together, as in the example cited here (Fig. 2). In other cases, the two terminals are many microns apart and may cross the striola. In the examples traced thus far, however, the polarizations of the cells communicating with the nerve are not in opposite directions.

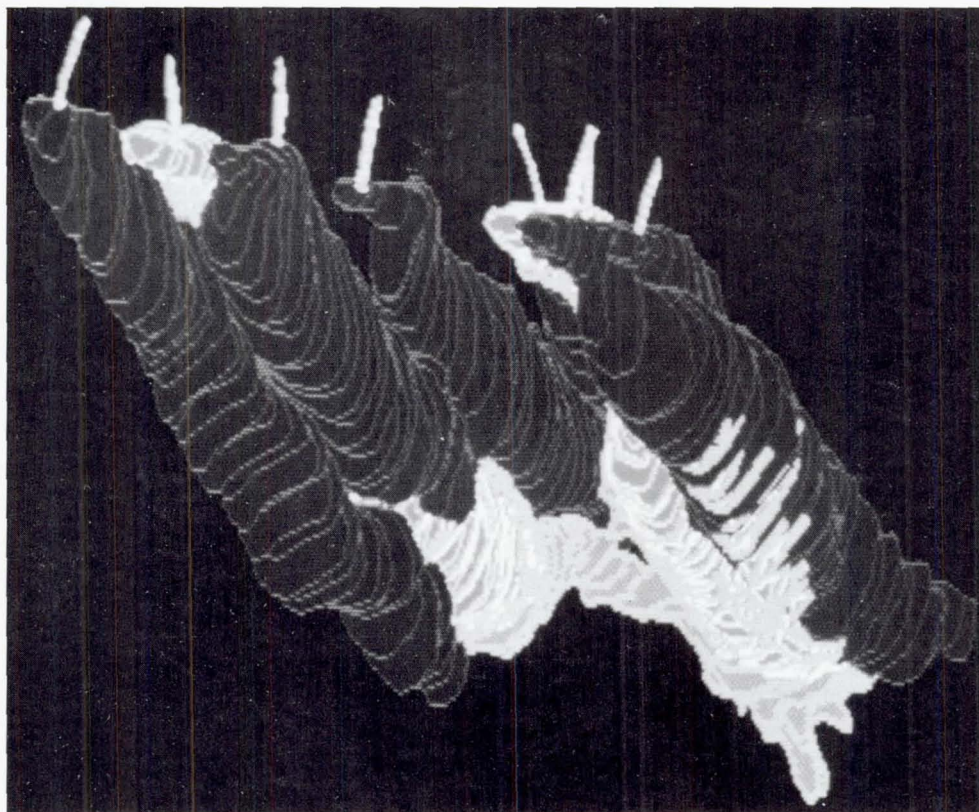


Fig. 2. This reconstruction illustrates a U-type pre-terminal with two unequal branches. One branch has a large calyceal collateral (afferent type) to a type II cell (far right). Each branch has 3 type II cells synapsing with it. Type I cells are blue; type II cells are red; kinocilia are purple and the calyx is yellow.

Neural Network Organization

The organization of the linked terminal fields can be illustrated by 3-dimensional reconstructions, but these rapidly become too large for a small computer to handle. The next stage of this portion of the reconstruction research, which has already begun, entails moving the data points to a workstation capable of more complex reconstructions and rendering them as solids, transparencies, or as slices through the entire field, from various angles.

Another approach is to begin to diagram the linked terminal fields as weighted neural networks, making them interpretable as electric circuits. By weighting is meant, for example, the number and distribution of cells synapsing with specific terminals of the network, the degree of similarity or disparity in angles of polarization of the cells of the sensory field, and the number, kinds and locations of synapses. These factors help determine the direction of information flow in the circuit at any given moment, depending upon the direction and force of the applied linear acceleration.

For the initial stage of this work, we diagrammed two parts of the lateral region of the utricular macula as neural networks, using hair cell number, distribution and linkages. The two parts were selected because of interesting terminals, some of which had been reconstructed. In the first network, connectivity between 67 cells was traced and in the second, 78. A representative part of each network is shown in Figures 3 and 4.

The networks from the two parts of the region examined are different in organization although they proved to be connected. In network 1, all three kinds of nerve/terminal innervation patterns were often linked together (Fig. 3). Surprisingly, network 2 consisted of only U-type nerve/patterns (Fig. 4). There were other interesting differences as well, which also have meaning in neural network theory.

First of all, the ratio of type I to type II hair cells is 1:1.5 in network 1, and 1:2.35 in network 2. Upon examining this finding more closely, it is clear that the 1:1.5 ratio holds because the calyces usually enclose 2-5 hair cells while 2-7 type II hair cells synapse with them along their exterior surfaces. In the case of the 1:2.35 ratio, the calyces enclose fewer type I cells (1-2), but the number of type II cells synapsing with them is most commonly 5. A further extension of these observations is that the average total number of cells synapsing with a terminal varies according to the type of terminal field in network 1. For example, for the region diagrammed, this number is 4 for U-types, 6 for M/U types, and 8 for the M types. In network 2, in which there are only U-type patterns, the average number of cells per terminal is 6.

The results gathered so far indicate that the smallest number of cells in a terminal field is, with rare exception, 4 (one instance of 3 has been found so far). No instances have yet been uncovered in which a single hair cell is innervated by a terminal.

Another observation is that type II hair cell clusters of the region studied here are larger and more predominant in network 1, where they might consist of as many as 7 cells. In network 2, interdigitation of more than two type II cells is uncommon (Figs. 3 & 4).

Finally, the two networks differ in another fundamental way. In network 2, the unmyelinated pre-terminals and their calyces often closely appose one another so that the intercellular space approximates that of a synapse. Such close apposition of these neural structures was not observed in network 1. Close apposition of neural surfaces, including those between type II hair cells, may signify electrical coupling to synchronize activity.

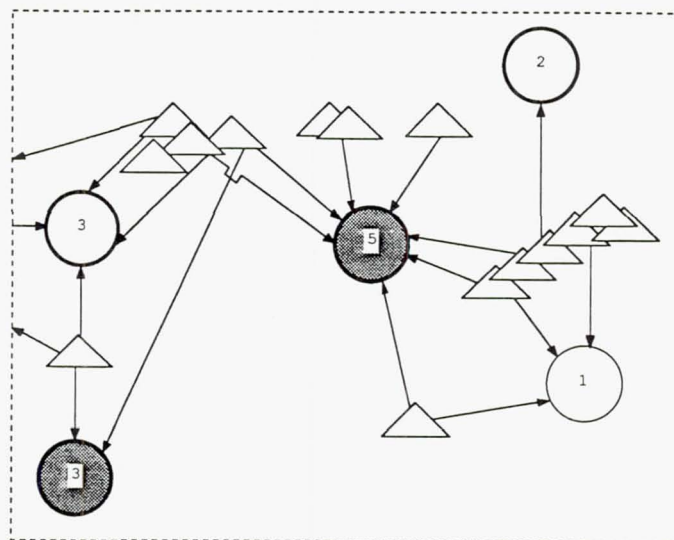


Fig. 3. This diagram is a representative piece of network 1 circuit diagram. Network 1 has all three nerve types linked, large clusters of type II cells, and a type I to type II ratio of 1:1.5. Type II cells are represented as triangles, calyces as circles with the number of contained type I cells noted, and adjacency as arrows.

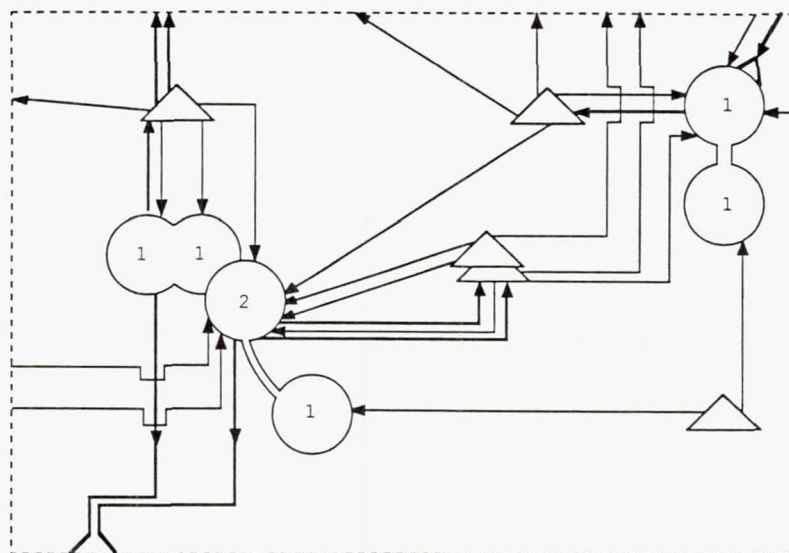


Fig. 4. This diagram is a representative piece of network 2 circuit diagram. Network 2 has only U-type pre-terminals, has close apposition of pre-terminals and calyces, and has fewer and smaller clusters of type II cells than does network 1.

As the research advances in technological capability, further information will be encoded in the circuit diagrams to better illustrate weighting. Noting the distribution, kind and number of synapses will be an important advance. For example, the number of synapses counted in cells reconstructed was 75, with rods prevalent in type I cells and spherules predominant in type II cells. This number does not represent the total junctions present, because only every fifth section was used for reconstruction purposes. Three-dimensional reconstructions using every section will be necessary to study this aspect of weighting in the neural circuits.

Nevertheless, sixty-one of the 75 synapses were in type II cells and, of this number, 50 were spherules. There were five examples of double junctions and serial or reciprocal junctions were not uncommon. Interestingly, 7 of the 14 synapses counted in type I cells occurred in the M-type field illustrated in Figure 1. These numbers emphasize that, in keeping with the high number of interconnections required for distributed processing, type II cells have many more synapses than type I cells. They also demonstrate that there may be weighting differences in synapses according to kind of terminal field. Lastly, they point to some need to know whether there are physiological differences between rod and spherule forms of synapses. One report indicated that spherule junctions are sensitive to catecholamines, at least in frog (Osborne and Thornhill, 1971), suggesting differences in sensitivity to neuromodulators.

Conclusions

The morphological observations clearly indicate that macular bioaccelerometers have a very sophisticated organization into weighted neural networks. The networks are in continuity with one another but differ in functional organization according to location. It is possible that further patterns will emerge as the study is continued to other macular parts. The reason for the relative complexity of some regions compared to others is unclear. The end organ only has to resolve direction and magnitude of linear accelerations; why are differing kinds of networks required to accomplish the job?

One possible answer is that there is enormous redundancy built into this natural parallel processor, so that it will remain a functioning unit even though parts of it are lost through attrition. Redundancy may be a hallmark of natural neural networks that could help explain their long lives, efficiency and adaptiveness.

A related question is why some nerves are regularly firing and some are irregular (Goldberg *et al.*, 1985). There is no precise relationship between calyceal or dimorphic units, the terms employed by Goldberg and his colleagues, and regularity or irregularity of discharge (Goldberg *et al.*, 1985). It would seem, however, that a plausible explanation exists if one studies the composition and organization of the neural networks described here. There is a potential for electrical coupling between calyces, between calyces and pre-terminals, and between pre-terminals in network 2 that is lacking in network 1. This could contribute to the smoothing out of neural responses in the unmyelinated nerves that intertwine, so that they are regularly firing. Because nerve pre-terminals and terminals of network 2 lack such close anatomical relationships, even though some U-type innervation patterns are included, their nerves would follow the patterns of firing initiated by the sensory/terminal network.

The findings presented here, then, argue for the concept that the morphological organization of the nerve innervation patterns and the relationships between components of the neural network are important in determining firing patterns. This point of view differs from that of Goldberg *et al.* (1985), who propose that postsynaptic recovery functions are a major determinant.

Because maculas are neural networks, it is clear that new approaches to the study of their physiology will be required. Means should be found to study nerve activity in adjacent nerves, and in nerves sharing information with the same type II hair cells. Advances in development of multi-channel electrodes, particularly if they will also permit injection of different dyes for later tracing of terminals, will be important. It must be kept in mind, however, that linked nerves do not necessarily enter the neuroepithelium adjacent to one another, particularly in the case of pars externa. A further, most critical need is for reconstruction of terminals of nerves of known physiological response, to learn whether the relationship between hair cell polarizations and specific directional sensitivity (Loe *et al.*, 1973) is linear.

It is clear that much more co-ordinated research involving morphologists, physiologists, physicists, electrical engineers, and neurochemists will be required to factually resolve the issues raised. In particular, a common terminology must evolve that will have clear meaning for the morphologist, physiologist, and neural network theoretician.

It is in this sense that a concerted NASA effort could be most beneficial to the vestibular community, by organizing and supporting an integrated effort to understand information processing in the vestibular receptors through appropriate ground-based research that will lead to a computer model of the weighted neural networks. NASA has the computer ability and expertise to make a significant contribution to neural network theory as well as to vestibular understanding, if its technological capability can be harnessed to a dedicated, investigative, research community. Such a concerted attack will be required if we hope to understand processes underlying neural adaptation to conflicts in signals induced by novel sensory environments, including space, or the unique gravitational fields of the moon and Mars. The findings should have the side benefit of providing a rational basis for devising counter-measures for space adaptation syndrome and for impaired vestibular function that may be particularly debilitating to those humans returning from long voyages in space.

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MARINE JOURNAL

ORIGINAL PAGE

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BLACK AND WHITE PHOTOGRAPH

JELLYFISH

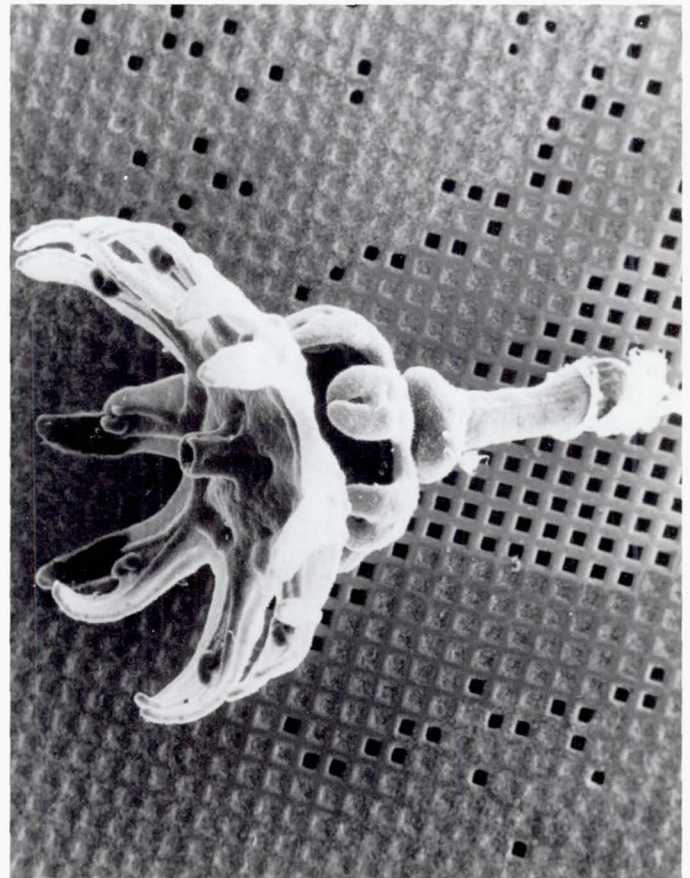
*Special Tools for Biological Research
on Earth and in Space*

By **DOROTHY B. SPANGENBERG**
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Eastern Virginia Medical School

When I was a youngster beachcombing in Galveston, Texas, I often saw large numbers of jellyfish washed up along the beach. I viewed the jellyfish, then, as most people do today, as nuisances. They cluttered the sand, often obscuring the more interesting seashell specimens I was collecting, and, of course, in general, they fouled the beach. It was years later, while studying at the University of Texas, Austin, that I began to realize what extraordinary creatures the jellyfish are and what great tools for biological research they could be.

My serious studies of the jellyfish, *Aurelia aurita* (moon jellies or plates) began in 1962 using a strain collected at Corpus Christi, Texas. I learned that the small polyp form of the *Aurelia* can be reared easily in small dishes of sea water (or artificial sea water) while being fed newly hatched brine shrimp (*Artemia salina*). Given good care, which involves changing the polyps into clean dishes and sea water after each feeding (now being done in my laboratory by ODU student Suzanne Davis), the jellyfish polyps will live indefinitely while continuously reproducing through budding. Indeed, I still have some of the Texas strain of jellyfish collected so many years ago!

The most intriguing nature of the jellyfish polyps, to me, is their ability to metamorphose, giving rise to tiny immature medusae called ephyrae which have a different form or shape from the polyps. In order to study the process of metamorphosis in the jellyfish, we had to be able to induce animals to metamorphose on command. After many years of research, we achieved this purpose by discovering that the jellyfish needed iodine in order to make a thyroid-type hormone required for metamorphosis. Today, we can "make" as many ephyrae as we need for our research studies whenever we need them by administering iodine and controlling their environmental temperature. This ability to control metamorphosis of polyps with iodine treatment led to the development of an exciting new test system, the *Aurelia* Metamorphosis Test System.



A nearly mature ephyra is developing at the top of a metamorphosing polyp. Less mature ephyra is underneath it. This is magnified approximately 50X.

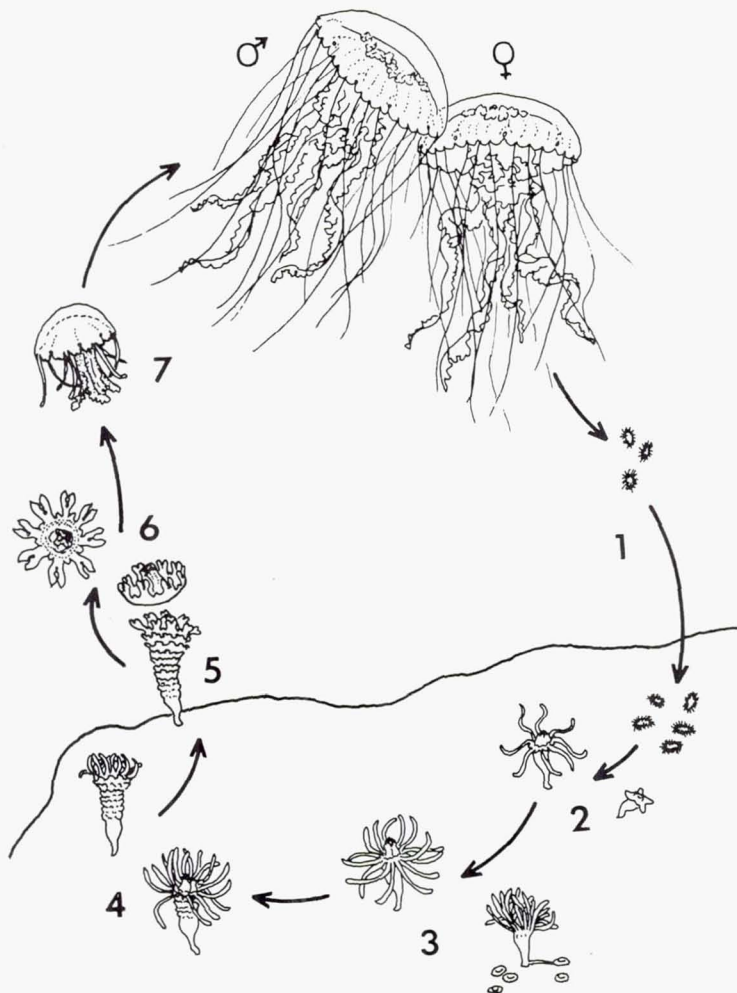
To date, we have used the *Aurelia* Metamorphosis Test System to determine the subtle effects of hydrocarbons found in oil spills (sponsored by the Department of Energy) and the effects of X-irradiation on developing ephyrae (sponsored by Eastern Virginia Medical School and done in collaboration with Mr. Mike Prokopchak). Currently, we are using this test system to determine the effects of the gravity-less environment of outer space on the development and behavior of ephyrae (sponsored by NASA). For this purpose, I am studying the effects of clinostat rotation on development of the ephyrae and their gravity receptors; we are looking at the behavior of the ephyrae during 0 gravity achieved for short intervals of 30 seconds in parabolic flight (in collaboration with Dr. Charles Oman, Massachusetts Institute of Technology); and we are planning exposure of developing ephyrae and of mature ephyrae to the gravity-less environment of outer space via a six or seven day shuttle experiment.

People ask me, "Why should we fly the jellyfish in outer space?" I reply that the jellyfish ephyrae form simple gravity receptor structures which resemble, in a less complicated way, the gravity receptor structures of higher organisms, including humans. The gravity-less environment of outer space is still a new, relatively unexplored frontier. We do not know, yet, how this gravity-less environment will affect the development of biological organisms or their gravity receptor structures. Indeed, we do not know what role gravity may be playing in our own development on earth. By comparing development of the ephyrae in space with that of ephyrae on earth, we expect to be able to answer questions about the importance of gravity which would be impossible to answer without the availability of the shuttle to take the jellyfish and other organisms into outer space.

It's a long journey for the jellyfish from the depths of the ocean to outer space, yet the jellyfish polyps and ephyrae are especially suited for the trip. They are tiny, require little or no care during their week-long journey, and have the capability of forming the special gravity-sensing structures. If the gravity-receptors do not form in outer space, we will deduce that gravity was needed for normal development (having controlled for other factors) and that gravity plays an important role in the normal development of these structures on earth. If gravity receptors do form in outer

space, we will study them in detail using various types of microscopes, including the electron microscope, to determine whether they developed normally in space as compared with controls on earth.

When I walked the beach of Galveston and avoided the jellyfish washed up there, I knew nothing about jellyfish metamorphosis or even that jellyfish made gravity-sensing structures. Indeed, the first space craft had not yet gone into outer space. Through basic science research of the jellyfish over the years, however, we learned about the special features of the jellyfish which make them especially valuable for gravity-related research. Today, when I walk the beaches of Norfolk and Virginia Beach, I think about the vast numbers of unexplored organisms in the ocean (and on the beaches) and I wonder about their special features and how they could be used to answer basic fundamental questions about living organisms. What a wonderful opportunity for scientists to have the use of such a smorgasboard of exotic marine animals with such a wealth of special features for exploration. Who knows what important questions will be answered through these aquatic organisms about life on earth and in space today and in the future!



Jellyfish life cycle, above, and drawing of comb jelly on page 4 are from *Common Jellyfish and Comb Jellies of North Carolina* by Frank J. Schwartz and illustrated by

Leslie Barling. Copies are available for 50¢ by writing P.O. Box 809, Moorhead City, NC 28557.

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EFFECTS OF CLINOSTAT ROTATION ON AURELIA STATOLITH SYNTHESIS

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Aurelia ephyrae develop eight graviceptors (rhopalia) during their metamorphosis from polyps, which are used for positional orientation with respect to gravity.

In three experiments for each speed of 1/15, 1/8, 1/4, 1/2, 1, and 24 rpm, groups of six polyps were rotated in the horizontal or vertical plane (control) using clinostats. Other controls were kept stationary in the two planes. Ten ephyrae from each group were collected after 5-6 days at 27°C in iodine and the number of statoliths per rhopalium were counted. Statistical analyses of statolith numbers revealed that horizontal clinostat rotation at 1/4 and 1/2 rpm caused the formation of significantly fewer statoliths per rhopalium than were found in controls. The finding that these slow rates of rotation reduces statolith numbers suggests that the developing ephyrae were disoriented with respect to gravity at these speeds, causing fewer statocytes to differentiate or to mineralize.

INTRODUCTION

Using the *Aurelia* Metamorphosis Test System (Spangenberg, 1984), we have been investigating the effects of clinostat rotation in the horizontal plane on the development of ephyrae and the synthesis of their statoliths.

Aurelia polyps are especially suited for gravity-related research because they are very small (2-4 mm), form ephyrae with gravity sensing structures (rhopalia) in 5-6 days, and can be used for clinostat studies. During iodine-induced metamorphosis (Spangenberg, 1967), ephyrae develop in sequential order from the oral to the aboral end of the polyps. Eight rhopalia with sacs of statoliths at their distal ends form per ephyra. These statoliths are composed of calcium sulfate dihydrate (Spangenberg and Beck, 1968) and only one statolith forms per statocyte.

METHODS

Two clinostats, made according to the design of Tremor and Souza (1972), were used for these studies. Jellyfish polyps were impaled head

downwards on cactus spines embedded in paraffin in the conical bottoms of autoanalyzer capsules (Figure 1). The capsules were filled with 10^{-3} M iodine in artificial sea water (ASW) prepared according to Spangenberg (1967). The caps of the capsules were filled with paraffin to eliminate bubble formation in the capsules, and the cap-capsule junction was covered with pressure sensitive tape to prevent leakage of the solution or evaporation. The capsules were tightly held in a 9 inch long glass tube with plastic joiners and the tube was attached to the shaft of the clinostat.

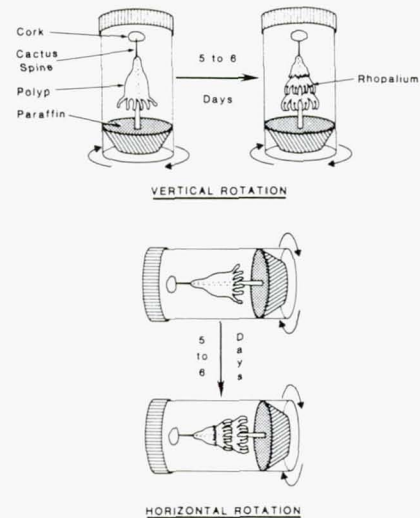


Figure 1. Orientation of the polyps and developing ephyrae during clinostat rotation.

Three tests were done for each clinostat speed using groups of 6 polyps (one per capsule) and speeds of 1/15, 1/8, 1/4, 1/2, 1, and 24 rpm were used. For each test, polyps were (1) rotated in a horizontal plane; or (2) rotated in the vertical plane; or (3) kept stationary in the horizontal position and placed near to (1); or kept stationary in the vertical position and placed near to (2). After 5-6 days at 27°C, the polyps formed ephyrae in all of the groups, and the ephyrae were removed from the spines in the capsules and squashed in a wet film. The excess ASW was removed to flatten the animals, causing the statoliths to spread, so that the number of statoliths per rhopalium per ephyra could be counted and recorded (Figure 2). Statistical analyses were done on the number of statoliths formed per rhopalium using an ANOVA and the Duncan's New Multiple Range Test.

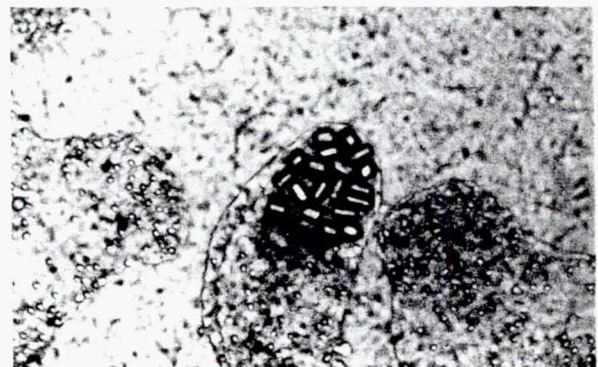


Figure 2. Statoliths spread in ephyra squash preparation.

RESULTS

Comparison of the numbers of statoliths formed by ephyrae which had developed during clinostat rotation in the horizontal plane with controls were made using an ANOVA which revealed significant differences between the groups at the $p < 0.01$ level and the Duncan's New Multiple Range Test. The latter test showed that those ephyrae which developed during rotation in the horizontal plane or 1/4 or 1/2 rpm had significantly fewer statoliths than controls which were rotated at these rates in the vertical plane or were kept stationary (Table 1). Ephyrae rotated in the horizontal plane at the other speeds of rotation did not have significantly different statolith numbers than controls.

Table 1. Numbers of statoliths in ephyrae which developed during clinostat rotation. (120 ephyrae in 3 tests per rotation speed).

Duncan's New Multiple Range Test				
Rotation Speed (rpm)	Clinostat (Horizontal Plane)	Clinostat (Vertical Plane)	Control (Horizontal Plane)	Control (Vertical Plane)
1/15	25.7	25.6	26.3	23.8
1/8	22.0	19.9	19.2	17.0
1/4	*20.2	25.1	25.0	27.3
1/2	*22.7	25.4	25.5	24.5
1	22.0	16.9	20.9	17.4
24	32.7	27.6	30.5	29.6

*Mean numbers of statoliths per rhopalium of ephyrae rotated in the horizontal plane which are significantly different from controls. $p = 0.01$

DISCUSSION

A variety of developmental and maturational effects have been found in plants and animals following clinostat rotation in the horizontal plane (Miquel, 1984; Brown, 1984; and Walgemuth and Grills, 1984). Clinostat effects have been found using both slow and fast clinostat rotation. The reduced statolith formation in the jellyfish ephyrae rotated at 1/4 and 1/2 rpm is a response to slow clinostat rotation. Other biological systems responding to slow clinostat rotation in the horizontal plane include: the formation of more developmental anomalies in *R. pipiens* and *X. laevis* following rotation of their egg at 1/4 rpm (Tremor and Souza, 1972); disturbed tobacco protoplast regeneration by rotation at 4 rpm (Iversen and Baggerud, 1984); and the delay of flower formation, seed production, and seed maturation which was found by Hoshizaka (1984) following rotation of *Arabidopsis* following clinostat rotation at 1/4 rpm.

A feature common to clinostat studies is the finding (as in the jellyfish statolith research) that rotation of organisms at some clinostat speeds elicit biological responses whereas no response occurs following rotation at other speeds. Lyon (1979) while referring to his experiments with *Coleus* and tomato plants reported that the difference in rotational time require-

ments for organs with different patterns of growth and hormonal controls illustrates the impossibility of setting a fixed rule for optimal rate of rotation of a clinostat. Iverson and Baggerud (1984) were unable to decide from their experiments which type of clinostat (fast or slow) provides the best simulated microgravity environment. These authors concluded that the response of their organisms indicated them to be a sensitive tool for studies of gravity effects which should be further tested in a real microgravity environment. Gruener (1984) also concluded that "it is impossible at present to assess the fidelity with which clinostat rotation simulates zero hypogravity encountered in space". Brown (1984) compared biological responses of plants rotated on the clinostat with those exposed to the microgravity environment of space. He found that circumnutation in plants occurred more vigorously in space than on the earth-based clinostat.

The mechanisms through which clinostat-rotation in the horizontal plane reduces statolith numbers in developing ephyrae is not known. In other organisms, such rotation has been reported to: cause mixing of intracellular constituents in frog eggs (Tremor and Souza, 1972); reduce movement of an auxin in *Coleus* and tomato plants (1970); significantly alter the functional interactions between the elements of a prototypic synapse in cultured cells of *X. laevis* (Gruener, 1984); cause a decrease in the content of starch grains in chloroplasts in protoplasts of tobacco using the slow but not the fast clinostat and to produce minor differences between protein patterns of rotated protoplasts and controls (Iversen and Baggerud, 1984).

Statolith synthesis in rhopalium of *Aurelia* ephyrae appears to be sensitive to disorientation of the organisms with respect to gravity. It is, therefore, possible that statolith synthesis will also be affected by exposure of developing ephyrae to the microgravity of space. Indeed, changes occurring in rhopalium following development in space could provide information which can lead to the identification of mechanisms through which microgravity affects statolith synthesis and through which gravity influences normal development of statoliths and rhopalium on earth.

ACKNOWLEDGMENTS

The authors are grateful to Dr. C. Ward for the loan of a clinostat and to Dr. T. Hoshizaka for supplying several clinostat motors. This research was funded by NASA, grant #NAG 2-343.

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Metabolism of Nonessential ¹⁵N-Labeled Amino Acids and the Measurement of Human Whole-Body Protein Synthesis Rates¹

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ABSTRACT Eight ¹⁵N-labeled nonessential amino acids plus ¹⁵NH₄Cl were administered over a 10-h period to four healthy adult males using a primed-constant dosage regimen. The amount of ¹⁵N excreted in the urine and the urinary ammonia, hippuric acid and plasma alanine ¹⁵N enrichments were measured. There was a high degree of consistency across subjects in the ordering of the nine compounds based on the fraction of ¹⁵N excreted (Kendall coefficient of concordance $W=0.83$, $P < 0.01$). Protein synthesis rates were calculated from the urinary ammonia plateau enrichment and the cumulative excretion of ¹⁵N. Glycine was one of the few amino acids that gave similar values by both methods. *J. Nutr.* 116: 1651-1659, 1986.

INDEXING KEY WORDS ¹⁵N • nonessential amino acids • protein synthesis

We are interested in the use of ¹⁵N-labeled glycine as a tracer for determining the human whole-body protein synthesis rate. There are several variants, the principal ones being 1) methods based on the enrichment of the urinary ammonia (1,2), 2) decay curve analyses based on the decay of isotope enrichment in the urine or plasma following a single pulse, (3-5), 3) from the cumulative excretion ¹⁵N excreted (6-8) and 4) a primed constant infusion-flux method (9).

Although several other methods involving ¹³C-labeled amino acids have been described, the methods using ¹⁵N are particularly suitable for use outside a hospital or clinical research center. The principal advantages of ¹⁵N are: 1) ¹⁵N can be used for single dosage-single sampling point methods, 2) serial sampling is not required and 3) there is no need to collect and analyze exhaled breath. Although other ¹⁵N-labeled amino acids have

been used, for example, alanine (10), aspartate (11) and lysine (9, 12), glycine is the most frequently used ¹⁵N-labeled amino acid because of its availability and relatively low price (13).

This study was designed to determine whether any of the other nonessential amino acids labeled with ¹⁵N offer any advantages over glycine. We compared the glycine-based protein synthesis rates (PSR) using both the urinary ammonia plateau, ¹⁵N enrichment and a cumulative excretion method against those found with other ¹⁵N-labeled nonessential amino acids. We also compared the relative effectiveness of glycine in transferring ¹⁵N to plasma alanine, urinary ammonia and urinary hippuric acid.

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MATERIALS AND METHODS

The subject population consisted of four healthy adult males between 27 and 42 years of age. Informed consent was obtained in accordance with the policies of the Graduate Hospital and the University of Pennsylvania, Philadelphia, PA.

Eight ^{15}N -labeled nonessential L-amino acids plus ^{15}N ammonium chloride were tested. ^{15}N glycine (99%) and arginine (96%) were obtained from US Services/ICONS, Summit, NJ; ^{15}N alanine (99%); $^{15}\text{NH}_4\text{Cl}$ (99%) and ^{15}N glutamic acid (99%) from KOR Isotopes, Inc. (now ICN), Cambridge, MA; ^{15}N aspartic acid (98%), ^{15}N serine (99%) and ^{15}N amido-labeled asparagine (99%) from Merck and Co., St. Louis, MO. and ^{15}N amido-labeled glutamine (99%) from Cambridge Isotopes, Inc., Cambridge, MA. Chemical and isotopic purity were verified by gas chromatograph-mass spectrometry (GC-MS). The amino acids were converted to their N-acetyl isopropyl derivatives, and the spectra were compared with the appropriate spectra from unlabeled compounds.

The total dose of ^{15}N ranged between 20 and 100 mg ^{15}N per subject. For the more expensive amino acids, the first subject took between 35 and 70 mg ^{15}N . The results were then analyzed and a decision made as to the minimum amount needed to give accurate data, and that dose was given to subsequent subjects. The reason for doing so was to minimize the cost of the study. The total dose (20–100 mg ^{15}N) was divided into 13 equal aliquots and placed in gelatin capsules. Each capsule also contained 300 mg of sodium benzoate. Glycine is conjugated with benzoic acid in the liver to give hippuric acid, which is excreted in the urine. Thus the urinary hippuric acid provides a means of sampling the hepatic glycine pool (14).

The nine ^{15}N -labeled compounds were given in random order at approximately one per month to the four subjects. The study was started at 0700, after an overnight fast, with the subject voiding and taking a priming dose of four capsules. The remaining nine capsules were taken at hourly intervals thereafter. Each time the subject took a cap-

sule he drank 50 mL of Ensure (Ross Laboratories, Columbus, OH). Ensure is a defined formula diet containing 1 kcal/mL. The 50 kcal/h of Ensure was given because this caloric intake (500 kcal/10 h) approximated the subjects' normal caloric intake for the period 700 to 1700. Subjects were permitted to consume noncaloric beverages (water, coffee or tea but with no cream or sugar) and nothing else from 0700 to 1700. At 1700, 7.0 mL of blood was drawn. Subjects collected a pooled urine for the period 0700 to 1230, after which time urine was collected approximately hourly until 1700. During the 10 h of the study the subjects performed their normal duties, which consisted of a combination of laboratory and office work.

Analytical methodology. Two methods were used for ^{15}N analysis. Plasma alanine enrichments were determined by GC-MS, and optical emission spectroscopy was used for the urinary nitrogen, ammonia and hippuric acid and the plasma urea. The sensitivity of the GC-MS and optical emission methods are ± 0.2 and ± 0.02 atom percent excess, respectively, in the range studied. Optical emission is the simpler technique, but requires a larger sample (3 μg of N_2) than the GC-MS method. Briefly, optical emission spectroscopy involves the irradiation of N_2 gas in a sealed 6-mm Vycor glass tube (Corning, Inc., Corning, NY) at 3 mmHg pressure with radio frequency energy. The N_2 molecules absorb the energy and reemit some of it as mauve light. The intensities of the emitted light at 297.68 nm ($^{14}\text{N}_2$) and 298.29 nm ($^{15}\text{N}^{14}\text{N}$) are compared by a standard monochromator-photomultiplier arrangement. This method requires that all samples be converted to N_2 gas, and this is usually accomplished by conversion to ammonia first and then oxidation to N_2 by alkaline hypobromite (15).

The blood urea nitrogen (BUN) was determined by the urease method using Sigma diagnostic kit #640 (Sigma Chemical Co., St. Louis, MO). For determination of the isotopic enrichment of the BUN, water (1 mL) and urease solution (2.0 mL, 0.21 mg urease/mL in 0.1 M phosphate buffer, pH 6.5) was added to plasma (2.0 mL). After incubating for 30 min at 37°C, K_2CO_3 (5 mL)

and 2-octanol (8 drops) were added. The ammonia was removed by aeration and collected in 0.1 N H₂SO₄ (1 mL). Total urinary nitrogen was measured on 1 mL of urine by the Kjeldahl method. Urinary ammonia was isolated from urine (5 mL) by adding saturated K₂CO₃ (5 mL) and aerating was done as described above for the BUN. The ¹⁵N enrichment of the urinary ammonia, total urinary nitrogen for each void (Kjeldahl distillates) and ammonia from the urease reaction on the BUN were determined by optical emission spectroscopy as previously described (15, 16).

Hippuric acid was isolated from the urine by crystallization after adjusting the urine concentration to 30% with sodium chloride (16). Occasionally the hippuric acid did not crystallize out. For those samples the urine (5 mL) was adjusted to pH 1 with concentration. H₂SO₄ and the urine extracted three times with ether (5 mL). The ether extracts were combined and evaporated to dryness. The pale straw residues from either the salt precipitation or the ether extractions were dissolved in minimal hot water. On cooling at 4°C, white needle-shaped crystals of hippuric acid separated out. The hippuric acid was then converted to (NH₄)₂SO₄ by Kjeldahl oxidation and analyzed for ¹⁵N by optical emission spectroscopy as described above.

The enrichment of the plasma alanine was determined by converting the plasma amino acids to their *N*-acetyl isopropyl esters as previously described (15). GC-MS analysis was done on a Hewlett-Packard 5992A GC-MS in the selective ion monitoring mode (SIM, ref. 15). The fragments at 186 amu (atomic mass units) and 187 amu were monitored. The parent peak is at 186 amu.

Methods of calculation. Fraction of ¹⁵N excreted. The amount of the administered dose excreted (**e*) was calculated by summing the ¹⁵N excreted in each urine sample collected over the 10-h period and the ¹⁵N remaining in the body urea pool at 10 h. The latter was calculated from the blood taken at the end of the experiment. The size of body urea pool (in grams) was estimated from the BUN (in milligrams/100 mL) using an equation derived by Hume and Weyers (17):

$$\text{urea pool} = \text{UDS} \times 10^2 \times \text{BUN}$$

where UDS is urea distribution space and $\text{UDS} = (0.195 \times \text{ht in centimeters}) + (0.297 \times \text{wt in kilograms}) - 14.013$. Thus ¹⁵N in urea pool = UDS (in liters) × BUN (in grams/liter) × BUN ¹⁵N (atom percent excess) × 0.01.

Whole-body protein synthesis rates. The whole-body protein synthesis rate (PSR) was calculated from the urinary ammonia ¹⁵N enrichment at plateau (18) and from the total amount of ¹⁵N excreted (6–8). From the urinary ammonia, PSR was calculated as follows:

$$Q = *d/A \text{ and } Q = E + S$$

where *Q* is nitrogen flux in grams N/hour; **d* is rate of ¹⁵N glycine administration in grams N/hour; *A* is ¹⁵N abundance in the urinary ammonia at plateau (APE·0.01); *E* is rate of N excretion in grams N/hour; *S* is rate of protein synthesis in grams N/hour; and APE is atom percent excess ¹⁵N.

From the total ¹⁵N excreted (cumulative excretion), PSR was calculated as:

$$S = E_T \cdot (*d/*e - 1)$$

where **d* is amount of ¹⁵N given in grams N 10/hour; **e* is amount of ¹⁵N excreted (urine + BUN) in grams N 10/hour; *E_T* is amount of N excretion in grams N 10/hour; and *S* is rate of protein synthesis in grams N 10/hour.

The total amount of ¹⁵N excreted (**e*) was defined as described above. In this study, we assumed that there was no change in the BUN during the course of the study, and the amount of ¹⁵N in the tissue free amino acid pools at 10 h was negligible (5, 19).

RESULTS

A one-way, repeated-measures design analysis of variance was run on each measure: 1) % of ¹⁵N excreted, 2) PSR by a cumulative excretion method, 3) PSR from the urinary ammonia ¹⁵N plateau, 4) urinary NH₃ ¹⁵N enrichment at plateau, 5) plasma alanine ¹⁵N enrichment and 6) the urinary hippuric acid ¹⁵N enrichment. The results are summarized in figure 1 and table 1. The Kendall coefficient of concordance (*W*) was determined for each of the above measurements to provide a measure of the degree of consistency across subjects ("intersubject reliability") for the amino acids (table 1, ref.

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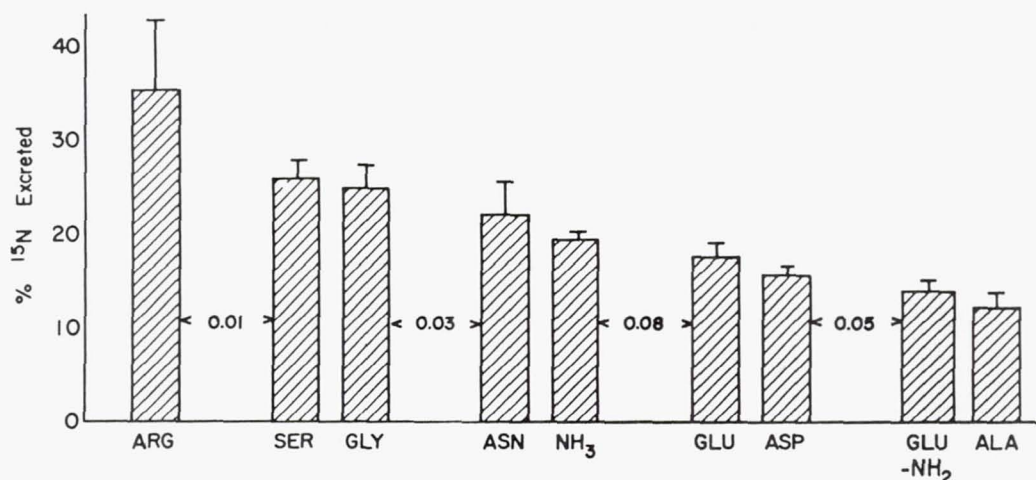


Fig. 1 Percentage of ¹⁵N excreted from various nonessential amino acids. Data is mean \pm SEM, $n=4$. Numbers between groups are P values.

20). The major findings of this study were:

1) There were significant differences in the fraction of ¹⁵N excreted among the various amino acids. The nonessential nitrogen compounds studied appeared to fall into five well-defined groups (figure 1).

2) There was high intersubject reliability in the ordering of the nine compounds based on the fraction of ¹⁵N excreted ($W=0.83$,

$P < 0.01$). All of the other ¹⁵N parameters except alanine also showed high intersubject consistency among subjects (table 1). As expected, parameters reflecting unlabeled nitrogen, such as nitrogen excretion and the BUN showed poor intersubject consistency ($W < 0.35$).

3) There was significantly less transference of ¹⁵N from guanido-labeled arginine

TABLE 1

Summary of synthesis rates calculated by flux (PSR_F) and cumulative excretion (PSR_T) methods and ¹⁵N enrichment of the urinary ammonia, hippuric acid and plasma alanine¹

	PSR _T	PSR _F	Urine hippuric acid ¹⁵ N	Urine ammonia ¹⁵ N	Plasma alanine ¹⁵ N
	g protein kg per d			APE	
Arginine	2.4 \pm 0.7 ^a	27.6 \pm 7.5 ^a	0.05 \pm 0.02 ^a	0.06 \pm 0.01 ^a	0.67 \pm 0.15 ^{b,c}
Serine	4.4 \pm 0.6 ^{a,b}	6.6 \pm 4.5 ^{b,c}	2.03 \pm 0.26 ^b	0.26 \pm 0.06 ^b	0.71 \pm 0.29 ^{a,b}
Glycine	4.3 \pm 1.0 ^{a,b}	5.2 \pm 1.9 ^{c,d}	1.93 \pm 0.31 ^b	0.23 \pm 0.06 ^b	1.71 \pm 0.89 ^a
Asparagine	4.8 \pm 2.1 ^{a,b}	9.2 \pm 2.2 ^b	0.12 \pm 0.01 ^f	0.16 \pm 0.13 ^c	0.91 \pm 0.69 ^{a,b}
NH ₄ Cl	7.2 \pm 2.7 ^{b,c}	13.2 \pm 2.5 ^a	0.26 \pm 0.02 ^d	0.11 \pm 0.02 ^c	0.58 \pm 0.08 ^c
Glutamate	6.2 \pm 1.2 ^b	7.2 \pm 1.9 ^{c,d}	0.26 \pm 0.04 ^{c,d}	0.19 \pm 0.03 ^b	0.89 \pm 0.10 ^{a,b}
Aspartate	6.9 \pm 1.9 ^{b,c}	10.3 \pm 4.5 ^{a,b}	0.23 \pm 0.02 ^{d,e}	0.16 \pm 0.05 ^c	0.83 \pm 0.11 ^{a,b}
Glutamine	9.4 \pm 2.7 ^c	5.1 \pm 3.1 ^d	0.17 \pm 0.02 ^e	0.25 \pm 0.05 ^b	ND
Alanine	10.4 \pm 3.3 ^c	9.2 \pm 2.4 ^b	0.35 \pm 0.02 ^c	0.15 \pm 0.03 ^c	0.99 \pm 0.42 ^a
Mean	6.1 \pm 0.9	10.4 \pm 2.6	0.60 \pm 0.28	0.23 \pm 0.05	1.07 \pm 0.34
W (P)	0.79 (0.02)	0.72 (0.05)	0.97 (0.01)	0.84 (0.01)	0.42 (NS)

¹ Values are means \pm SEM. ND, no data. Unlike superscripts differ by $P < 0.05$. APE is atom percent excess (¹⁵N). W is the Kendall coefficient of concordance (20).

to ammonia or hippuric acid than from any other compound (table 1).

4) Two of the subjects excreted a very large proportion of the guanido N from arginine as urea (48 and 44 % vs. 23 and 21 %).

5) Less ¹⁵N from alanine and glutamine were excreted than from any of the other compounds tested (fig. 1).

6) Reasonable urinary ammonia ¹⁵N plateaus were obtained for the nine compounds (figure 2).

7) There was no close relationship between the protein synthesis rate based on the urinary ammonia ¹⁵N enrichment (PSR_F) and the value derived from the total amount of ¹⁵N excreted (PSR_T).

DISCUSSION

Selection of study conditions. The normal dietary intake between 0700 and 1700 of the four subjects ranged from breakfast (toast and coffee) and a meal for lunch to black coffee only. The dietary regimen (50 kcal/h) of Ensure was a compromise designed to approximate the mean normal nutritional intake of the four subjects for that period. During the study, the subjects performed their normal daily duties.

As a proportion of the oral diet given, the test amino acid may have represented up to a 50 % increase in the dietary amount of the test amino acid. Such relatively high dosages are routinely used in stable isotope tracer studies because of the relatively high enrichment levels needed for detection with most currently available analytical equipment (9). The "tracer" amino acid is mixed with the much larger pool of amino acids derived from protein breakdown so that the contribution to the amino acid flux approaches a tracer dose (~5 %; refs. 9, 18, 19, 21).

Excretion of ¹⁵N. There was high inter-subject reliability in the ordering of amino acids based on the fraction of ¹⁵N excreted ($W=0.83$, $P>0.01$). Furthermore, the compounds tested appeared to fall into five well-defined groups (fig. 1): 1) arginine, 2) serine and glycine, 3) asparagine and ammonia, 4) glutamate and aspartate and 5) glutamine and alanine (fig. 1). These groupings correspond to 1) an amino acid which is a very

close precursor of urea (arginine), 2) amino acids which are interconvertible and metabolized via ammonia and one carbon transfer reactions (serine and glycine), 3) compounds that are metabolized as ammonia (NH₄Cl and asparagine, which is hydrolyzed to ammonia in the gut), 4) amino acids whose carbon skeletons play key roles in intermediary metabolism (aspartate and glutamate) and 5) dual-function amino acids where the carbon skeleton is involved in intermediary metabolism and interorgan nitrogen transport (glutamine and alanine).

Although it was expected that a large proportion of the nitrogen from arginine would be metabolized to urea, the magnitude in two of our four subjects was surprising (48 and 44 % vs. 21 and 23 %). This may be due to human variability (22). Arginine has been reported to be a marginally essential amino acid (23).

Transference of ¹⁵N to other nonessential amino compounds. A high degree of consistency across subjects in the ordering of amino acids was found for the ¹⁵N enrichment of urinary ammonia and hippuric acid but not for alanine (table 1), possibly because of the lower sensitivity of the GC-MS analysis (± 0.2 atom percent excess). Like the alanine and hippuric acid ¹⁵N enrichment, the urinary enrichment of ammonia is another indicator of ¹⁵N transference but reflects both the enrichment of the free amino acid pools and the proportion of ammonia derived from the carrier amino acid. No other amino acid transferred significantly more ¹⁵N to urinary ammonia or plasma than glycine (table 1).

Calculation of protein synthesis rates. Although all estimates of the human body protein synthesis rate are based on the three-pool compartmental model developed by San Pietro and Rittenberg (5), there are many variants, each of which is based on slightly different sets of assumptions (18, 19, 24, 25). From all of these methods a consensus has emerged that the daytime human whole-body PSR is in the range of 1.5 to 6 g protein/kg per d. In this study we obtained values from the urinary ammonia plateau ¹⁵N enrichment and from total amount of ¹⁵N excreted. There was no apparent relationship between the whole-body PSR values derived

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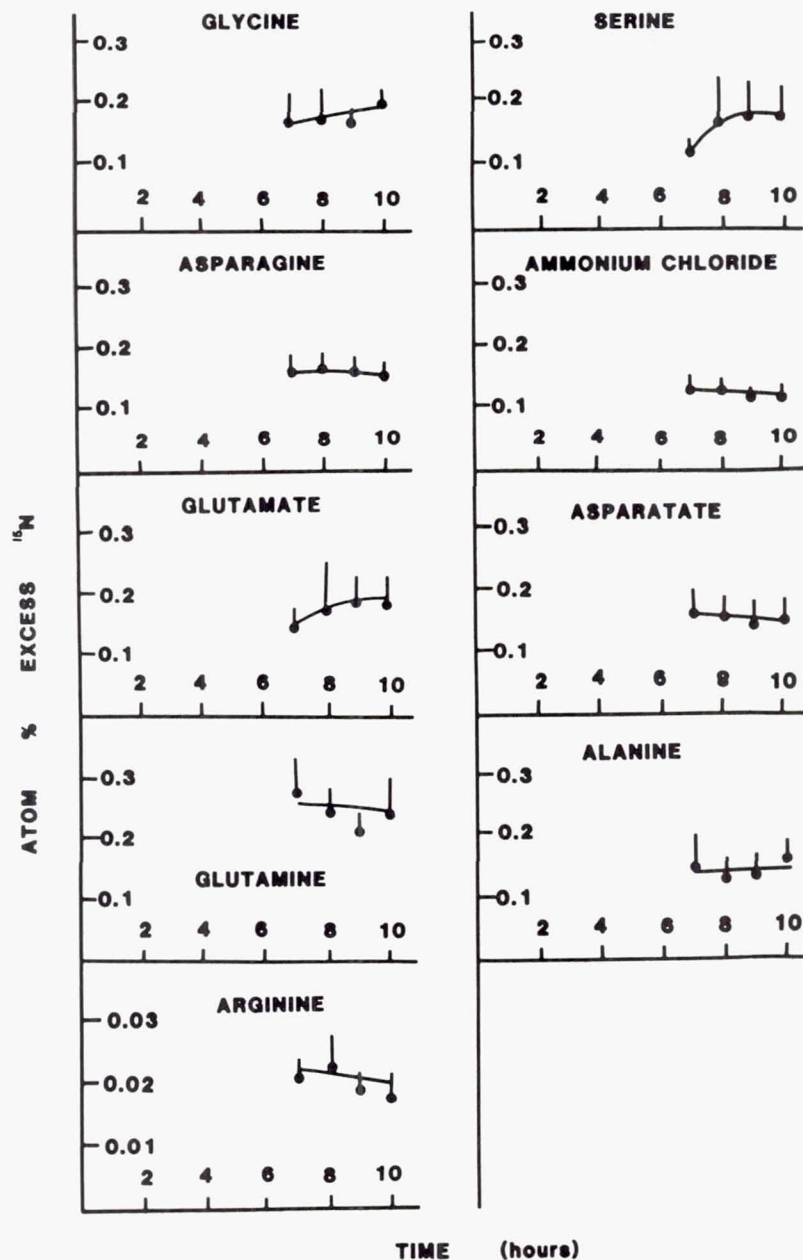


Fig. 2 Urinary ammonia enrichments for the urine specimens collected between 1230 and 1700. Data not normalized.

from the urinary ammonia and total excretion of ^{15}N .

Cumulative ^{15}N excretion. The basic assumption underlying the end point approach is that administered ^{15}N is parti-

tioned between end product (urea) and protein in the same proportion as total nitrogen, most of which is derived from endogenous protein breakdown. Although this issue has been addressed by several authors

previously, it is still not resolved for ^{15}N (7, 8, 13). The partitioning coefficient, or synthesis rates derived therefrom, should be independent of the route of isotope administration. Unless they are, one or both routes of isotope administration cannot reflect the composite endogenous nitrogen metabolism. Thus this study yields data on two criteria: 1) the calculated PSR should be in reasonable agreement with the value derived by other (carbon-labeled) methods, and 2) there should be reasonable agreement between the intravenous and orally derived values.

Most of the PSRs calculated by the cumulative excretion method in the present study are within the "consensus range" of 1.5 to 6 g protein/kg per d. The oral values found for glutamine and alanine (10.4 and 9.4 g protein/kg per d) in this study were high, although rates within the "consensus range" have been reported with the intravenous route (2.8 and 3.6 g protein/kg per day), respectively (10, 24). The oral aspartate and glutamate values are also elevated (table 1). These four amino acids are, as pointed out above, dual-function amino acids, and the second function can affect the partitioning of nitrogen. Previously, Tarunga (26) reported that essential amino acid nitrogen was unsuitable as a tracer because the nitrogen metabolism reflected the metabolism of the carbon skeleton. This study extends that conclusion to nonessential amino acids where the metabolism of the amino acid is dependent on both the partitioning between synthesis and excretion and the role in intermediary metabolism of the deaminated amino acid. Glycine meets the criteria of having no other major metabolic role if serine is regarded as part of the glycine pool. Glycine gives the same values orally or intravenously (25).

Urinary ammonia. There are at least two routes by which ^{15}N can be transferred to ammonia and then excreted in the urine. One is by entering, mixing and equilibrating with the body's free amino acid pools, a key assumption of both the flux and end product approaches. The other is by serving as a precursor to urinary ammonia without having attained isotopic equilibration in the tissue free amino acid pools. Different

amino acids contribute differently to the urinary ammonia, although the major precursor is plasma glutamine, and this may explain the lack of correlation between the methods (table 1, ref. 24). The values derived for the two methods were more similar for glycine than for any other ^{15}N compound tested.

The cumulative excretion method is much less dependent on the urinary ammonia enrichment because more than 90% of the ^{15}N is excreted as urea. These uncertainties, the lack of correlation with the more theoretically sound total-excretion-based values and the range and scatter in the flux-based values suggest that the cumulative excretion of total ^{15}N is the preferable approach.

Use of ^{15}N -labeled nonessential amino acids as tracers. There is some question as to how widely applicable single-isotope whole-body methods are. Anomalously high PSRs have been reported with glycine (8, 27), tyrosine (28) and leucine (29) in situations where there was hepatic insufficiency. The liver is particularly important in the mixing and equilibrating processes. Unexpectedly low (negative values) have been found with [$1\text{-}^{13}\text{C}$]leucine in human subjects on low protein diets and during a simulated triathlon (30, 31). The fact that apparently anomalous results are found with several tracers suggests that the effect is not specific to the amino acid used but a failure of the model where liver insufficiency or other serious perturbations may result in compartmentation of the tissue free amino acid pools (27). The presence of any of these situations limits the applicability of single-isotope whole-body methods for the measurement of human whole-body protein synthesis rates.

The use of [^{15}N]glycine. The PSR values derived from the two methods used in this study were closer for glycine than for any other amino acid and are similar to those found with ^{13}C -labeled amino acids. No other nonessential ^{15}N -labeled amino acid has been shown to be superior to glycine. [^{15}N]glycine has several distinct advantages for studying human protein metabolism: 1) Methods involving [^{15}N]glycine are technically the simplest (e.g., single-point assays (2)); 2) [^{15}N]Glycine is relatively cheap and readily available in a pure state; 3) More is

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known about the metabolism of [^{15}N]glycine than any other ^{15}N -labeled amino acid; 4) Only [^{15}N]glycine offers the possibility of simultaneously measuring liver-originated plasma protein synthesis rates by using the urinary hippuric acid to determine the enrichment of the liver intracellular glycine pool (16).

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A Systems Approach to the Physiology of Weightlessness

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and Carolyn S. Leach

This paper presents a systems approach to the unraveling of the complex response pattern of the human subjected to the challenge of weightlessness. The major goal of this research is to obtain an understanding of the role that each of the major components of the human system plays following the transition to and from space. The cornerstone of this approach is the utilization of a variety of mathematical models in order to pose and test alternative hypotheses concerned with the adaptation process. An integrated hypothesis for the human physiological response to weightlessness is developed.

INTRODUCTION

Research concerned with the physiological adaptation of man to the weightless environment of space is replete with special challenges. In the past, manned space flights have been relatively infrequent and expensive, with the result that only a small number of subjects have been available and the statistical significance of the observed changes has been difficult to assess. In addition, for valid and understandable reasons, the types of measurements made on man have been limited. Equipment used in the environment of space has had to be specially designed for each task, and the development and testing of major equipment has required long lead times. Finally, any observed physiological response in the normal astronaut population is actually the result of a combination or integration of the responses of several different body systems, each responding simultaneously to the challenges of space flight and weightlessness. The individual systems involved are themselves complex, containing both competing and redundant pathways and multiple interconnections.

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In the future, some of these problems related to space flight are likely to disappear. In the new Shuttle era, manned flights, in near Earth orbit, will be frequent, and the number of astronauts going into space will be considerably larger than in any previous space program. A large number of special life sciences experiments utilizing the Spacelab module will be conducted, and the diversity of the physiological measurements made will be greater than ever before. In preparation for the future flight opportunities, an inventory of special flight-tested life sciences laboratory equipment (LSLE) is currently being built. In effect, the ground rules are changing so that life sciences research in general, and human physiological research in particular, are becoming easier to conduct in the weightless environment of space.

This paper is concerned with the most difficult problem remaining in the study of man's adaptation to weightlessness and subsequent readaptation to the environment of Earth. This problem involves unraveling the integrated total human physiological response to obtain an understanding of the role that each of the major components of that system plays following the transition to and from space. In its basic form, this problem is not a new one in physiology or medicine. A similar problem is encountered whenever one subjects a complex system to a stress that affects several components of the system at once. This paper will present a general systems approach to conducting and analyzing research on the human adaptation to weightlessness. The cornerstone of this approach is the utilization of a variety of mathematical models in order to pose and test alternative hypotheses concerned with the adaptation process. This general approach will be illustrated by considering certain aspects of the problem of fluid and electrolyte shifts in weightlessness. Finally, an integrated hypothesis based on numerous simulation studies and available experimental data will be presented.

MODELING

Each of us has, through experience, built up a sense of what a model is. In effect, it is an image of reality that captures the essence of some aspect of form and function from something else. In the sciences, one's notions about a particular system often can be represented in symbolic terms, with real quantities and processes replaced by symbols and mathematical operations. The result of this representation is an axiom system that can be identified with what is called a mathematical model. Such a model is usually an imperfect image of reality, except in trivial cases. The discovery of the imperfections of a model can be time-consuming and fraught with difficulty because of the fact that most mathematical models of interest in the life sciences are nonlinear and involve coupled differential equations. In that case, heavy computational machinery is usually involved in extracting some of the consequences of the axiom system. This ties the use and development of major models in the life sciences to the more powerful computing systems. However, many of the simpler models can be studied effectively using the new generation of microcomputers,¹ and this advance should do much to popularize the use of such models, especially as teaching aids.

In science, mathematical models are built and studied for several reasons. The very act of constructing a model requires systematic, logical thinking, and generally provides new insight into the organization of the system elements, the processes within

the elements themselves, and the multiple pathways connecting the system elements. Model building leads almost immediately to the identification of gaps in the experimental knowledge of the system of interest and suggests particular ways of filling those gaps. In addition, models can provide information concerning the regularities and patterns of behavior that a system possesses. When a system is complex, such patterns can be hidden from view simply because one does not know what to look for. By clarifying what to look for, a model may predict both correlations and cause-and-effect relations that are subject to actual verification in the real system.

If one considers a model to be a collection, H , of hypotheses, then H is said to imply C , where C represents the collection of conclusions of the model. By observing C , or part of C , in the real system, one would like to turn things around and say that C implies H . In general, this is impossible, but by matching part of the predictions with actual data, one generates a level of confidence in the correctness of the hypotheses. This level varies with the size of the set C that is matched, and it is difficult to quantify. In practice, the most useful results are obtained when the observed C does not match the predicted C exactly. Analysis of the differences involved can then lead to a modification of the hypotheses that define the model, or to the need for new and clarifying experiments. Such a process is generally iterative, being applied over and over again with new stresses designed to force the model to display more and more of its weaknesses. Reconstruction of the model by modification of the hypothesis set leads to a stronger model, in some sense. As the model behavior moves closer to the behavior of the real system through iterative modification of the hypotheses, the probe of science sinks ever deeper into the subject. In fact, this iterative use of models is only a restricted form of the general scientific method. In the next section, this approach is formalized and specialized for the study of the physiological problems of weightlessness.

Each of the particular models used in this project is a deterministic, nonlinear representation of a major human physiological system. All models are implemented on a digital computer system using a finite difference formulation, and all are used in an interactive time-sharing mode with automated graphical output. The models, taken together, are able to provide simulations of a wide variety of physiological events spanning a large time frame, from seconds or minutes to months in length. The basic models in use are a pulsatile cardiovascular model,^{2,3} a respiratory model,^{4,5} a thermoregulatory model,^{6,7} an erythropoiesis regulatory model,⁸ a circulatory, fluid, and electrolyte balance model,^{9,10} and, a model of calcium regulation.¹¹ In addition, the first five of these models have been merged into a common framework termed the "whole-body algorithm,"¹² which has been used to simulate composite human function, particularly during space flight. The six basic models are illustrated conceptually in Figures 1 and 2, and the whole-body algorithm is shown in Figure 3. All models are characterized by an active controlling system that regulates a relatively passive controlled system, and, taken together, these two major components function as a (usually negative) feedback control system. The major feedback variables present in these models represent many of the actual sensors present in the body, including temperature sensors, chemoreceptors, baroreceptors, oxygen sensors, and osmoreceptors. The models were validated using a wide variety of experimental and clinical conditions, and a partial list of these validation studies is presented in Table 1. With all of these

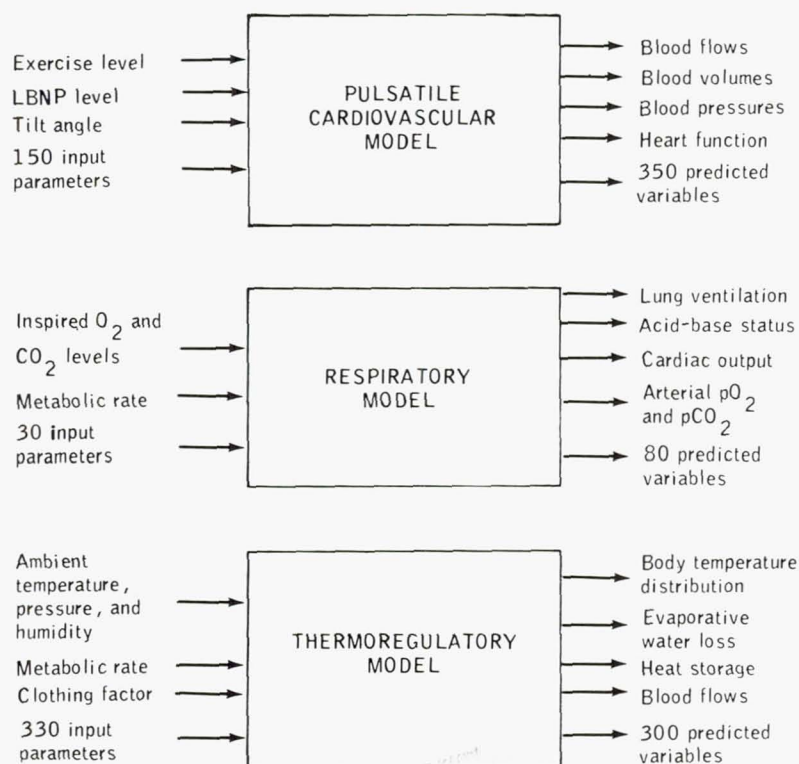


Figure 1. Models used for simulating short-term physiological events.

models, multiple stresses and sequential stresses of varying degrees can be applied, just as in an actual experimental protocol. A few typical simulations produced by some of these models will be discussed later in this paper.

Table 1. Stresses Related to Space Flight That Were Used to Validate the Responses of the Simulation Models

Hypogravic stress	Environmental disturbances
Supine bed rest	Hypoxia
Head-down bed rest	Hypercapnia
Water immersion	Temperature
	Ambient pressure
Orthostatic stress	Fluid shifts
LBNP	Hemorrhage
Tilt table	Infusion
Postural change	Water and salt loading
	Dehydration
	Metabolic stress
	Exercise
	Diet restriction

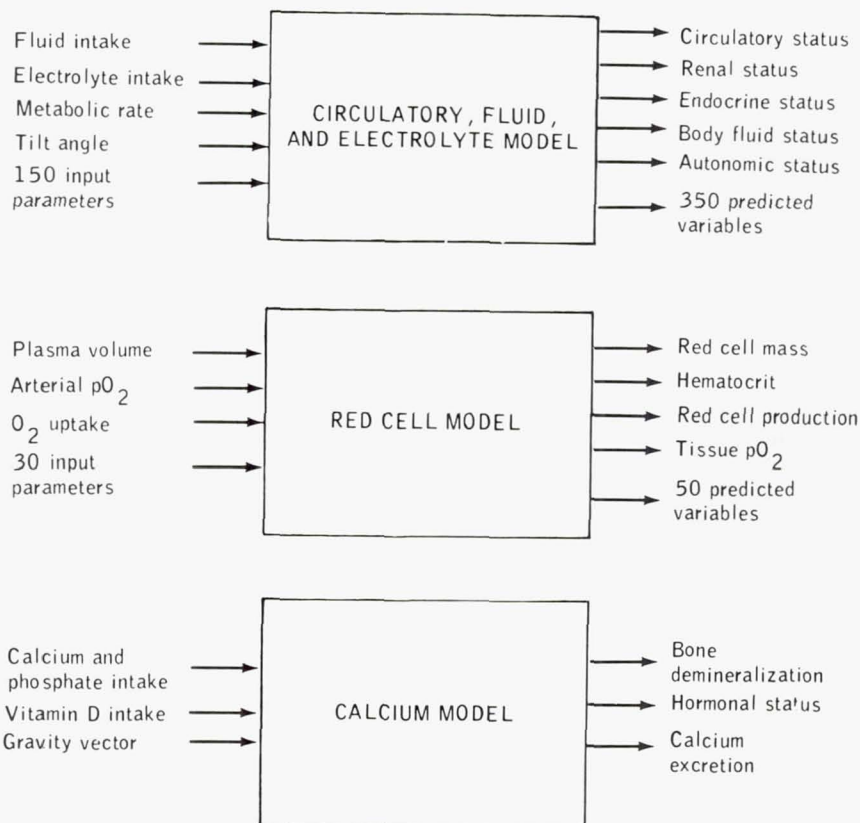


Figure 2. Models used for simulating long-term physiological events.

SYSTEMS ANALYSIS

Figure 4 presents the general systems approach designed for the study of the physiology of weightlessness. This restricted, but powerful, form of the scientific method has been implemented using the specially designed integrated medical data analysis system shown in Figure 5. The systems analysis approach is clearly iterative, and one may enter the main loop at any point. The automated data base and analysis system permits large arrays of space-flight and pertinent ground-based data to be scanned rapidly, while variables are correlated visually or automatically, and hypotheses are tested for statistical significance. Such an evaluation of experimental data can lead, under appropriate conditions, to a qualitative formulation of alternative mechanisms involved in producing the observed responses.

The next step, which requires an in-depth understanding of the capabilities and limitations of the relevant model or models in physiological terms, draws heavily on the theory of feedback-regulating systems and requires that the hypotheses in question be formulated in quantitative terms suitable for actual testing using one or more of the models available. The integrated medical data analysis system (Figure 5) was designed

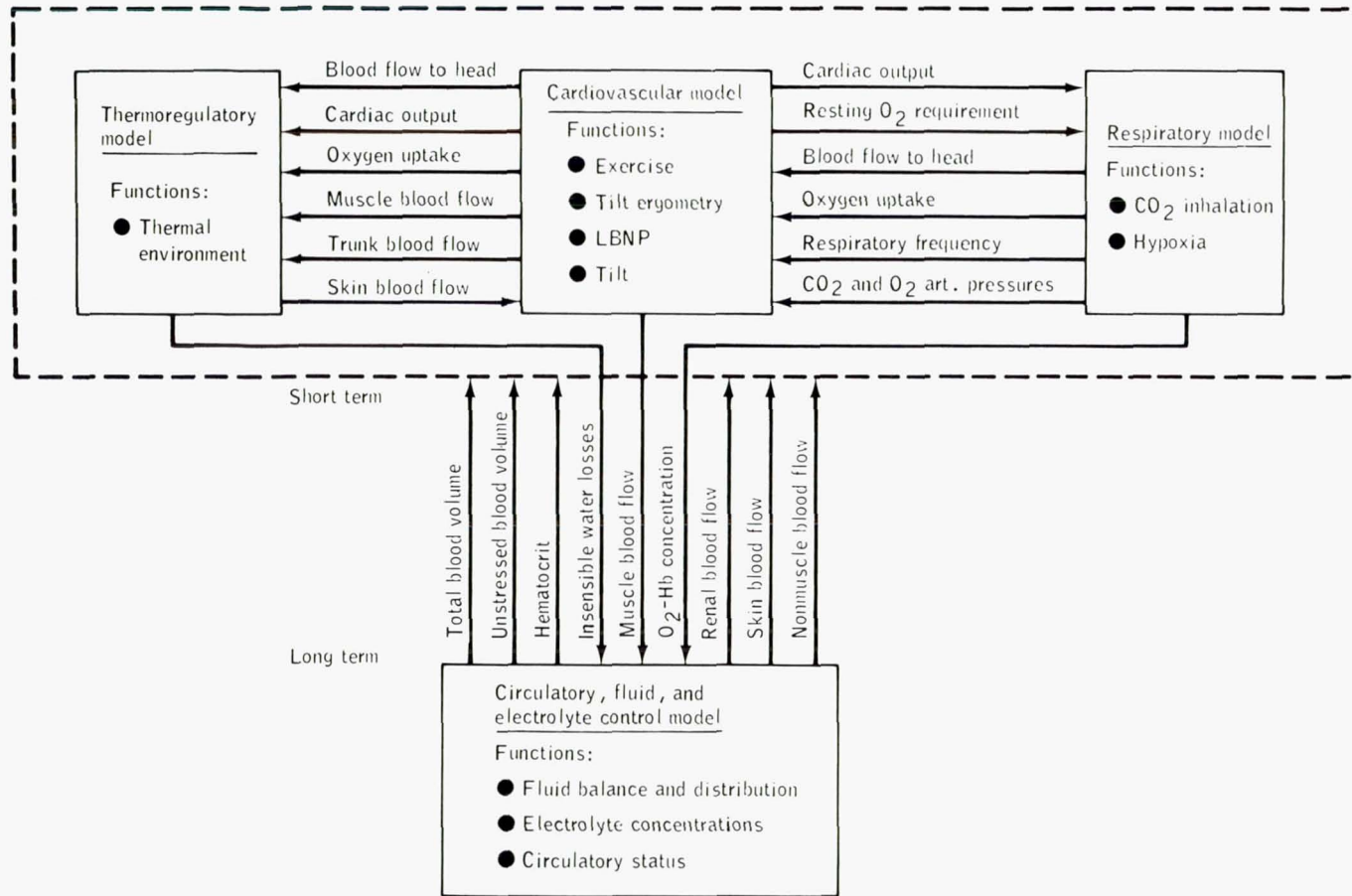


Figure 3. The whole-body algorithm.

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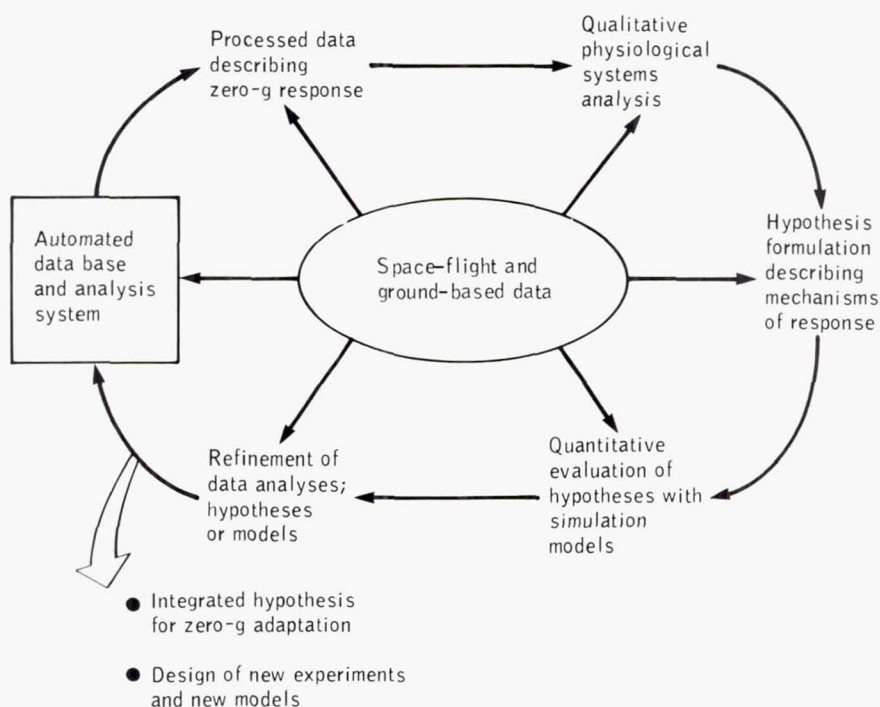


Figure 4. Systems analysis approach to understanding the physiology of weightlessness.

so that the elements could interact in either sequential or parallel fashion, enabling a portion of the actual experimental data to be employed as input (forcing) functions to a simulation model while the model output can be compared to another portion of the experimental data in the data base.

Actual testing of the hypotheses using the available models constitutes the next step in this process. Good agreement between model output and experimental data furnishes a certain level of confirmation for the hypothesis under consideration but does not "prove" that the hypothesis is true. As in almost all applications of the scientific method, there are only varying degrees of confirmation involved in the process, not absolute certainty. Poor agreement or contradictions between model output and actual data cause one to question the hypothesis under investigation, the structure of the model itself, or the validity of the experimental data. In actual practice, such a state of poor agreement, at least initially, is the more interesting one, and leads to a level of improvement in one of the above major elements. In this way, the modeling process contributes to an increased understanding of the system.

This whole process must be able to be applied consistently to man in space or on Earth during various maneuvers, since the same physiological systems are involved in both cases, at least initially. Some Earth-based studies are particularly relevant to the function of the body during weightlessness, and they have been used as experimental analogues of weightless space flight. These include water immersion, bed rest, and

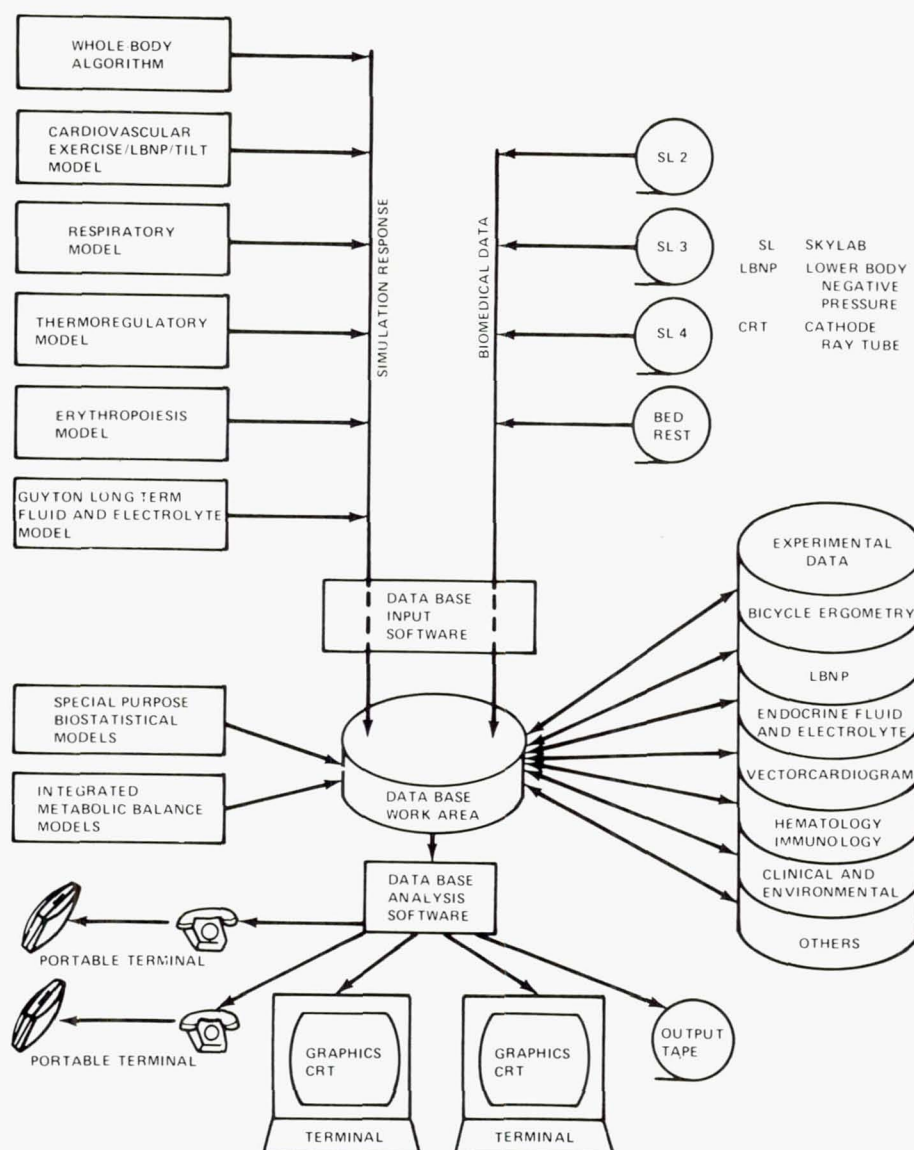


Figure 5. Integrated medical data analysis system.

head-down bed rest studies, and the modeling of such studies has been an important part of our overall analysis program.

RESULTS

A large number of simulations of a variety of types using all of the models mentioned earlier in this paper have been completed. Some of them have been reported

on previously²⁻¹⁷ and a book, which contains others, is currently in preparation. Reporting on even a fraction of these actual studies is outside the scope of this paper, and only a sample of this work is presented here. At the end of this section, an integrated hypothesis, combining the final result of many of the individual studies, will be discussed.

Responses of the body systems that regulate human fluid and electrolyte balance to the stress of weightlessness can be divided into at least two distinct phases. The acute phase is characterized initially by a reduction in the normal gravity-induced hydrostatic gradients and a rapid headward shift of fluid. Then, the body rapidly responds, through feedback, to relieve some of the stresses felt by the central portion of the circulation. This acute phase lasts for 1 to 2 days and is followed by an adaptive phase, which is characterized by much less dramatic change. As mentioned in the previous section, ground-based analogues of true weightlessness are particularly useful because of the limited information gathered on previous space flights and because of the fact that the same physiological systems are involved in the responses to the similar stresses. A mathematical model provides a framework to systematically examine the various hypogravic stresses and to study their similarities and differences. Simulations are presented of two of these analogues of true weightlessness in order to illustrate typical model capabilities.

Figure 6 presents the results of a simulation study of 5° head-down tilt in man using a modified version of the model of Guyton.⁹ This modified model has leg vascular and tissue compartments, gravity-dependent circulatory elements, and a natriuretic factor. At the break in the curves shown in Figure 6 ($t = 0$), the angle of body tilt (to the horizontal) was modified and the elastic forces in the tissues and vessels themselves redistributed the body fluids in a manner favoring upper-body hypervolemia. Ordinate scales are omitted in this figure to emphasize the qualitative aspects of changes pictured. Quantitative data are presented in Table 2. Subsequently, feedback controllers respond by regulating blood flow, blood pressure, and central blood volume toward more normal values. Autonomic, renal, and hormonal control elements all contribute significantly in this response. Table 2 compares the results of this simulation study with the actual data recently collected after 24 hours of such tilt by Blomqvist.¹⁸ Examination of this table and comparison of Figure 6 with an idealized figure presented by Blomqvist (his Figure 2) shows that the simulation presents a qualitatively and quantitatively accurate (for most Figure 6 variables, given normal variability) picture of the events involved in head-down tilt. The usefulness of this simulation will be addressed later when many such studies are integrated into a discussion of space flight.

Figure 7 presents a simulation study of water immersion in man. Here, fluid was shifted out of the lower part of the body by the application of external compression on the leg interstitial and venous compartments. The results of this maneuver are similar to, but not identical with, the head-down tilt study shown in Figure 6. Comparison of the water immersion simulation to average experimental data^{19,20} shows that the simulation results are representative of the typical response.

By comparing the results of simulation studies of head-down tilt, water immersion, lower body positive pressure, supine bed rest, and space flight, it is possible to determine the pathways and mechanisms in the model that play a determining role in

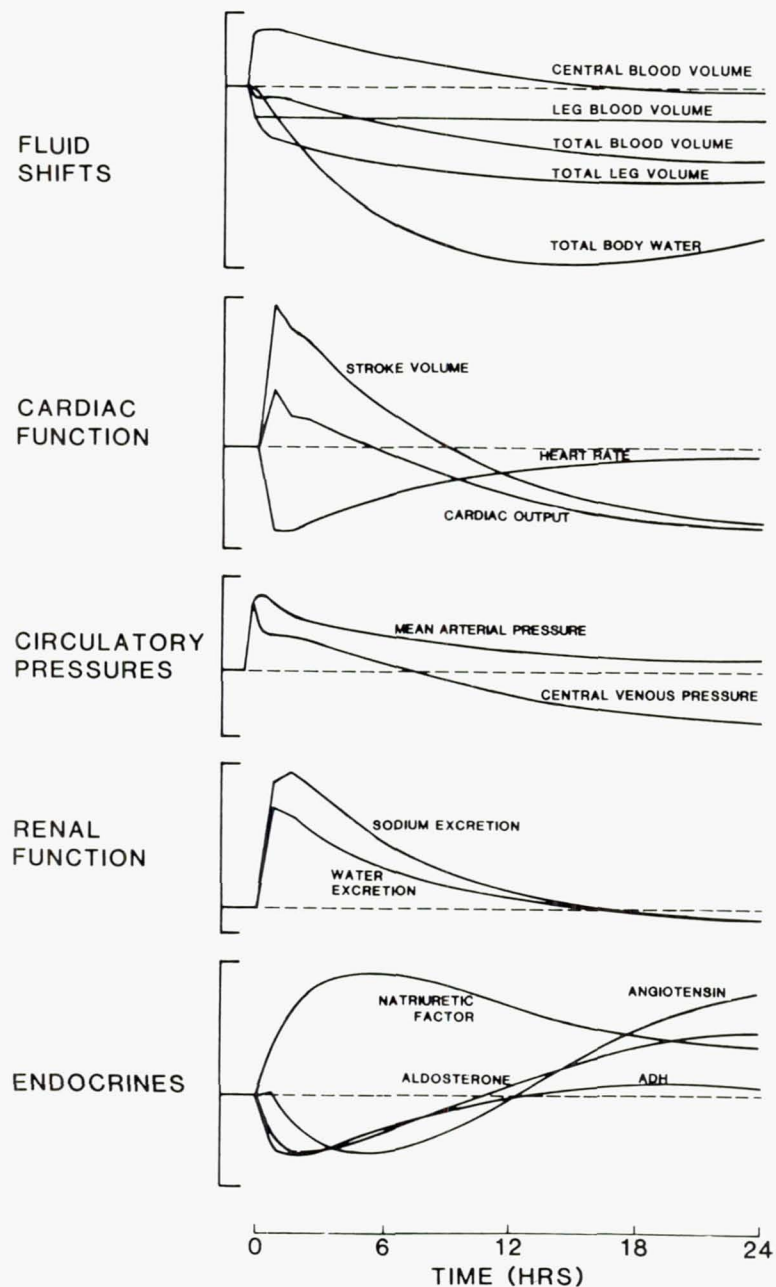


Figure 6. Simulation of head-down tilt.

each of these related stresses, and to suggest key experiments that can confirm these pathways and mechanisms in man. This has been done in the case of space-flight studies and has led to the proposal of several possible future Shuttle experiments. A major study of the acute aspects of fluid shifts during weightlessness will take place on

Table 2. Comparison of Model Output and Experimental Response for 24-Hour Head-Down (-5°) Tilt Study

Quantity	Value at hours compared to control	
	Experiment ¹⁸	Model
Fluid shifts		
Total body water	-1300 ml	-1130 ml
Leg blood volume	—	-256 ml
Leg interstitial volume	—	-454 ml
Leg volume	-900 ml	-710 ml
Total blood volume	-425 ml	-563 ml
Urine rate, 8 hr/24 hr	127%	190%
Hemodynamics		
Cardiac output	-7.8%	-11.1%
Stroke volume	-8.5%	-9.8%
Heart rate	0%	1.4%
Arterial pressure	+4%	+3%
Central venous pressure	-49%	-9%
Left atrial pressure	—	-50%
Hormones		
Aldosterone	+35%	+17%
Angiotensin	+25%	+27%
ADH	+57%	+3%
Natriuretic factor	—	+13%

the dedicated life sciences mission to be conducted by the United States in 1985.

Figure 8 presents an integrated hypothesis for the human physiological response to weightlessness. This hypothesis includes most of the major systems of the body. It was generated from the systems approach discussed earlier (Figure 4) through simulation studies using the models already mentioned. The experimental data, without which these modeling studies would have been impossible, are reported in several places.²¹⁻²⁴ Although a great amount of detail has been omitted from Figure 8 for the sake of clarity and simplicity, the broad interactions among many of the body's systems are presented in a unified fashion. The following picture has emerged. Disturbances in the cardiovascular, fluid-electrolyte, erythropoietic, musculoskeletal, and metabolic systems, which are found during and after space flights of varying duration, appear to be attributed to two major effects of weightlessness. These are, first, the absence of hydrostatic forces, resulting in severe fluid shifts within the body, and second, the absence of deformation forces, resulting in degradation of normally load-bearing tissues. The first of these effects leads to a reduction in body fluids—most important, blood volume. The major consequence of the second effect is a reduction in bone and muscle mass. In addition, a third factor, a long-term alteration of metabolic state, which itself reflects changes in dietary intake and exercise, was found to play an important role in the responses of the Skylab crews. All of these events have both acute and long-term effects that lead to the notable and consistent findings of loss in weight, change in body composition, decreased tolerance for orthostasis, and, upon return to a 1-g environment, a compromised response to physical activity. Adaptation is said to occur when the body adjusts to these changes and reaches a new steady-state level.

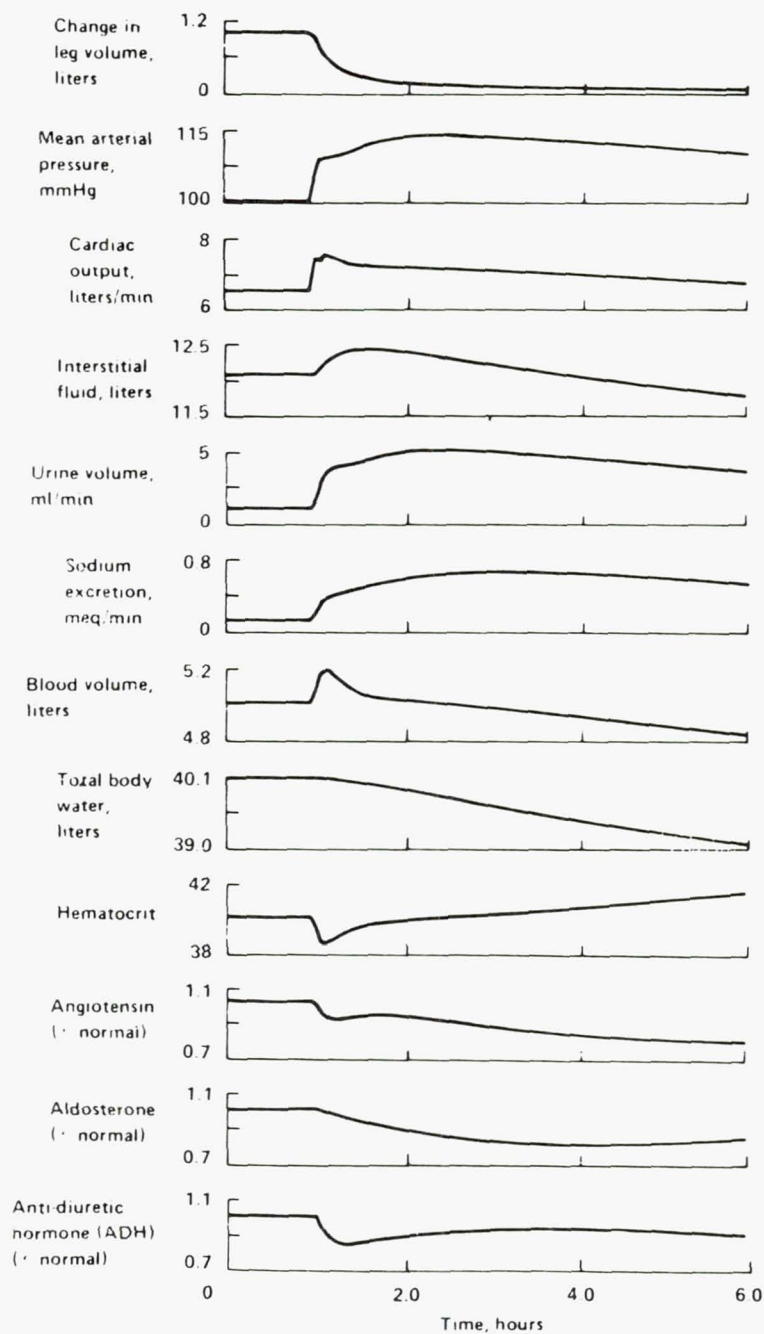


Figure 7. Simulation of water immersion.

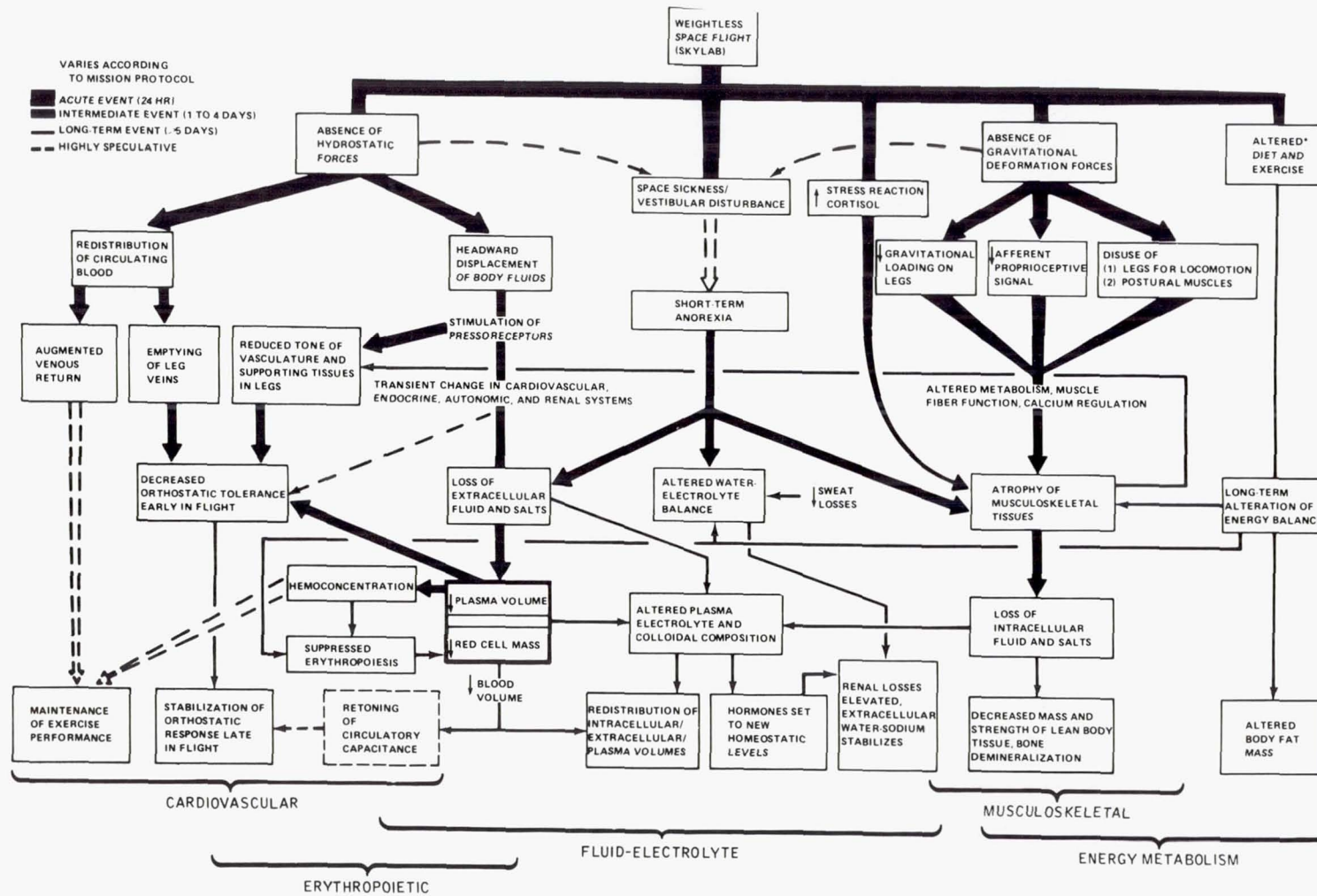


Figure 8. Integrated hypothesis of physiological adaptation to prolonged space flight.

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Figure 9 is an attempt to show the relative time course of adaptation for each major physiological system. The return to baseline reflects the establishment of a new homeostatic level appropriate to weightlessness.

Our simulation studies have supported the concept that, within the time span that man has so far been studied in space, these responses to weightlessness can be explained in terms of normal feedback regulatory processes. One classical example of such processes concerns the blood volume controllers that reduce plasma volume when challenged by the cephalic shifts of peripheral fluid. Also, the reduction observed in red cell mass has been postulated to be partly a result of the homeostatic response to hemoconcentration and tissue hyperoxia. Another of many examples concerns the biochemical mechanisms that sensitively respond to small changes in electrolyte shifts resulting from cell demineralization, and, within limits, maintain the plasma composition at the expense of excess renal excretion.

Exposure to weightlessness invariably leads to losses of major body constituents at rates that, according to the present analysis, are disproportionate to their concentrations in the body. The most rapid losses are observed for extracellular fluids and salts, and are reflected by equally rapid decrements in leg volume. At the other extreme is a class of substances that are lost by the body much more slowly. Calcium and perhaps red cells are representative of this category. Depending on the degree to which caloric intake matches energy requirements, fat stores can be included in this group as well. Muscle tissue appears to degrade at an intermediate rate, as exemplified by nitrogen and potassium losses. All these rates of disappearance from the body most likely depend on the nature of the disturbance, and on the effective time constant of the correcting homeostatic system.

Our simulation studies have confirmed the hypothesis previously suggested that the loss of blood volume is of central importance to the understanding of the zero-g responses of several major systems. While this loss is believed to be an acute circula-

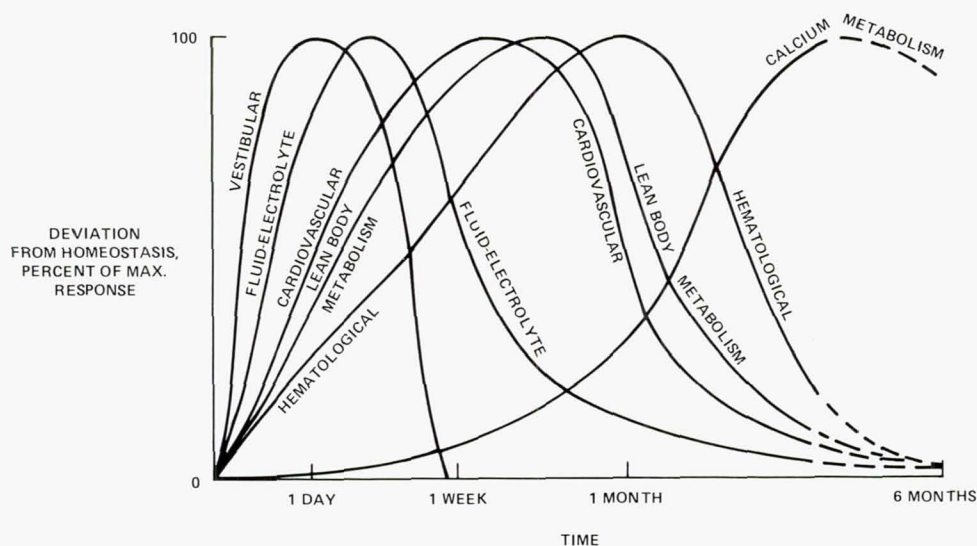


Figure 9. Approach toward homeostasis of physiological systems during space flight.

tory adaptation to volume overload, it was found to play a significant long-term role in the orthostatic intolerance and reduced exercise performance observed postflight. Also, the reduced plasma volume was found to have a potentially strong influence on the erythropoietic response. Finally, the long-term adaptation of the circulation may depend on the vascular elements responding to accommodate the hypovolemic condition. It should be noted, however, that in none of these cases was the loss of blood volume alone sufficient to explain the entire response. Another event that had a widespread effect was the negative energy balance noted for the crews of the two shortest Skylab missions and for all crews during the early flight period. In addition to the obvious effect on fat stores, an inadequate intake of fluid and food was found to be implicated in the lack of expected acute renal response, the loss of muscle tissue, the loss of water, and the differential loss of red cells among the crews.

CONCLUSION

The contributions of the systems analysis approach, in general, and the mathematical models, in particular, were invaluable in constructing the hypothesis discussed above. This hypothesis should be treated with appropriate caution, since some of the interconnections between the elements shown in Figure 8 are still tentative and have not been confirmed by direct experimental evidence. In spite of this limitation, these studies have led to an improved understanding of the physiology of weightlessness. When this understanding is even more complete, it should be possible to define appropriate indices of health (normal adaptation) during space flight and, using models, to predict individual responses to weightlessness. Combining these two elements, it should be possible to develop appropriate counter measures to the deleterious effects of space flight (upon return to Earth).

There are many additional benefits derived directly and indirectly through the systems approach using mathematical modeling, as sketched in this paper. For example, the models themselves serve as composite references to the present state of knowledge of the different physiological systems. As new information is obtained about the function or control of a particular system, that information can be used to improve or update the model in question. Thus, a model implicitly can contain the theoretical strengths and weaknesses of an entire field of study. Use of such knowledge can be an effective means of guiding or planning a research program, and one of the spinoffs of modeling and the systems approach concerns the area of research planning. A second additional benefit, only alluded to earlier, concerns the potential applicability of these same models to the study of abnormal states and, therefore, to clinical problems. At present, this application is one of the major challenges of the future.

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CHAPTER 22

Perception of the body in space: mechanisms

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THE PRINCIPAL TOPIC TREATED IN THIS CHAPTER is the perception of body orientation and motion in space and the extent to which these perceptual abstractions can be related directly to our knowledge of sensory mechanisms, particularly for the vestibular apparatus.

The relationship between physical variables trans-

duced by the sensory systems and human perception of orientation was still a subject of vigorous debate during the last century. The notion that perception could be discussed in terms of a machine acting on external variables was foreign to the basic ideas of Kant, which influenced nineteenth-century thinkers so much. The central role of the vestibular apparatus as the organ of equilibrium had not always been accepted, even after the demonstration of their importance in postural control (55). The long and unfortunate distinction between physical stimuli and sensory processes on the one hand and perception on the other hand has been reviewed by Boring (16) and by Teuber (152) and this distinction is placed in its historical perspective in the chapter by Jung in this *Handbook*.

Increasingly, sensory end-organ characteristics and central nervous system activity in response to complex multisensory stimuli are related to the perception of body movement reported by a human being under similar stimulus conditions. It is clearly appropriate to relate sensory-unit activity resulting from body-motion stimuli to such purposeful motor activities as head or eye stabilization. It is no less relevant to relate underlying sensory transduction and the higher processing of these afferent signals to the generation of motion perception. Long after it was fully recognized that we see with our eyes and hear with our ears, the origin of the perception of spatial orientation remained a mystery. At the beginning of the nineteenth century the sense of equilibrium was still judged to be related to shifting the fluid in the cranium as the head orientation was changed by gravity. Perhaps the most critical experiment in creating a sensory basis for spatial orientation was performed by Flourens (55). He demonstrated the essential role of the semicircular canals in postural stability and equilibrium and, incidentally, related their stimulation to the genesis of motion sickness. It remained for Mach, a physicist and natural scientist of the mid-nineteenth century, however, to relate the physical characteristics of the semicircular canals and the otolith system to the quantitative perceptual measures of tilt and rotation. In his

influential treatise, Mach (106) found it necessary to explain at length why motion sensation had to be associated with mechanical stimuli of the sensory organs. Remnants of this unfortunate dichotomy between stimulus and perception remain to the present time.

The next major thrust in the causal link between vestibular organ stimulation and perception of orientation came through demonstrations that involuntary eye movements, as a special case of sensorimotor reactions, were also driven directly by vestibular stimulation. The recognition that slow compensatory eye movements would result from direct cupula stimulation of semicircular canals (48) and later that ocular counterrolling, or compensatory torsion of the eye about the axis of gaze, could be attributed to otolith as well as semicircular canal stimulation greatly enhanced the ability of workers in the field of human spatial orientation to relate vestibular stimulation to perception and eye movements.

The first half of the twentieth century saw a growth of research activity devoted toward quantitative evaluation of the physical transduction properties of the vestibular organs, especially of the semicircular canals, based largely on measurements of human perception of rotation and tilt during deterministic transient motions or steady-state sinusoidal oscillations. The physical characteristics of the semicircular canals, clarified by the demonstrations of Steinhausen (147) and Dohleman (47) that the cupula does not normally allow endolymph to flow freely through the ampulla, were translated into differential equation form (148, 160). Recently it has been possible to make quantitative estimates (129) and actual measurements (88, 127) of cupula deflection and to relate them to rotation sensation for arbitrary rotation stimuli.

It is tempting but misleading to regard spatial orientation and postural reactions to environmental disturbances as automatic or reflex reactions. Certainly, many environmental disturbances lead to highly stereotyped postural reactions that direct an animal toward a desirable situation, away from danger, or toward the reestablishment of a stable posture. There was a period following the turn of the century, based on the theories of Loeb (104), when such orienting behavior was described as thoroughly mechanistic. Orientation, at least in the lower animal, was conceived of as being totally determined by, for example, the presence of light or the angle relative to gravity. Postural reactions and locomotion were seen as reflexes totally independent of sensation. Even for invertebrates this extreme view of mechanistic orientation behavior has been largely discarded (58). Such automatic, machinelike postural orientation reactions are seen in mammals only under special circumstances. Specifically, on decerebrating a cat the automatic righting reactions are easily seen. It is tempting to attribute orientation sensations for the intact animal to direct pathways from graviceptor stimulation

or asymmetric tactile stimulation. The actual perception of orientation, although relying on the various sensory inputs that also produce postural reactions, is heavily influenced by a number of cortical functions, including expectation of the input and habituation to a particular stimulus sequence.

The latter third of the twentieth century has seen a rebirth of interest in nonvestibular contributions to self-motion perception, especially the visually induced perception of motion occurring in response to a uniform moving field (86). These visual illusions of motion sensation, as well as the less-documented illusions of self-motion based on manipulation of tactile, proprioceptive, and auditory cues, lead to the current "systems view" of mechanisms of perception of the body in space. Under most circumstances the multiplicity of sensory inputs related to spatial orientation is processed by the central nervous system to produce only a single, usually nonambiguous, perception of body orientation and movement. The process by which these various sensory signals are reduced and compared with one another is known as multisensory integration. For example, the extent to which a given visual motion contributes to the perception of self-motion is influenced strongly by the concurrent vestibular stimulation or lack of it. Furthermore, all of the multisensory integration logic is plastic or modifiable. Perceptual, postural, and eye-movement responses to identical sensory patterns exhibit habituation to repeated stimuli and adaptation to rearranged sensory signals. Thus, for example, the normal responses of rotation perception, compensatory eye movements, and postural reactions to combinations of head movement and visual-field rotation are all greatly modified by sensory stimulus rearrangements as simple as wearing reversing prisms (63, 64, 125) or as complex as exposure to weightlessness. Furthermore, the systems view of this multisensory integration allows for the processing of sensory signals to be modified on the basis of the "expected response." This expected response might reflect either an efferent copy of active movement undertaken by the subject, which would normally produce a given feedback pattern, or it might reflect the expected continuation of a well-learned pattern of movement such as oscillation on a swing. This multisensory integration approach requires knowledge of the dynamic response of the various sensory organs associated with human spatial orientation as well as estimation of the physical situation in which the subject finds himself (14, 15).

PERCEPTION OF ORIENTATION BASED ON MULTIPLE SENSORY MODALITIES

A frequent problem in relating physical stimuli to perception is that the number of dimensions of the perception exceeds that of the stimuli. Teuber (152) pointed out that for audition, independent variations

of only frequency and intensity lead to variations in the perception of pitch, density, volume, and loudness. For motion sensation, however, the situation is quite simple: perceptions are limited to linear and angular position and body motion in three dimensions. Referring to the standard definition of body axes in Figure 1, the vector of orientation sensation includes tilt orientation with respect to the vertical (pitch and roll), angular heading orientation (yaw), their first derivatives (rotation rates), and possibly angular accelerations. Linear displacement (forward-backward, left-right, up-down) is a three-dimensional vector perceived as linear position; linear velocity and possibly linear acceleration are also sensed. Perceived spatial orientation is closely related to the actual linear and angular motions of the body, and many of the important differences between perception and true motion are explainable on the basis of the dynamic characteristics of the sensors. In marked contrast to studies of perception of sound or light, however, motion perception is usually based on simultaneous stimulation of one or more of several sensory systems. Visual, tactile, and proprioceptive stimuli, as well as vestibular inputs, can produce motion sensations by themselves and modify motion sensations in conjunction with other sensors. We do not distinguish among a visual motion sensation, a vestibular motion sensation, and a tactile motion sensation. Consequently, in discussing the mechanisms involved in motion perception it is not surprising that much of the emphasis is placed on the interaction among different sensory modalities rather than simply on the transfer characteristics from stimulus to sensation through a single sensory channel.

Motion perception is strongly influenced by mental set or the preconceived idea of the subject as to what types of motion are permissible or what the limits of the test apparatus may be. Active control by a subject

of his own body motion in space, whether through direct muscular activity as in postural control and locomotion or through command of a vehicle, may result in vastly different perceptions of motion than would be true for a passive observer receiving the identical sensory stimuli. Habituation to repeated presentation of the same sensory stimuli may result in a decreased perception of body motion.

Simultaneous processing of information from the vestibular, visual, proprioceptive, and auditory channels regarding human spatial orientation is illustrated schematically in Figure 2. The spatial orientation sense cannot be turned off by closing one's eyes or by eliminating acoustic stimuli. The absence of any head movements to stimulate the vestibular system is not sensory deafferentation but rather a definite signal indicating the continuation of constant-velocity motion (including zero velocity). Proprioceptive and tactile inputs can never be removed except during periods of free fall, considered in more detail as a special case of environmental adaptation in section SPATIAL ORIENTATION IN ALTERED ENVIRONMENTS, p. 1060. The perception of relationships between parts of the body, such as the estimation of limb position, is not discussed in this chapter except as related to the interpretation of visual or vestibular signals. The important influence of active motor control on both actual and perceived motion of the body is indicated in Figure 2. Furthermore, the expected patterns of motion, whether based on continuation of an existing pattern or on recollection of the motion from the previous exposure to the same situation, are of vital importance in determining the manner in which multiple sensory inputs are combined to yield a single perception of spatial orientation and movement. Special cases of ambiguous stimuli resulting in confusion about spatial orientation or of behavior that alternates between two or more different orientations may result in vertigo or motion sickness and are discussed separately in *Motion Sickness*, p. 1061.

Semicircular Canals

The semicircular canals, as described in detail in the chapter by Goldberg and Fernández in this *Handbook*, are fluid-filled rings that respond to angular accelerations having a component normal to the plane of the ring by deviation of the cupula and stimulation of the hair cells in the crista. As a result of their arrangement in three roughly orthogonal planes within each labyrinth, the semicircular canals are able to detect and transduce angular accelerations about any axis in space. Because of their narrow lumen and the large contribution of viscous force inside the tube relative to inertial forces, they act as approximate integrators. Consequently for all but very low frequencies of stimulation their output reflects angular velocity rather than angular acceleration of the head with respect to inertial space. They fail as angular-velocity trans-

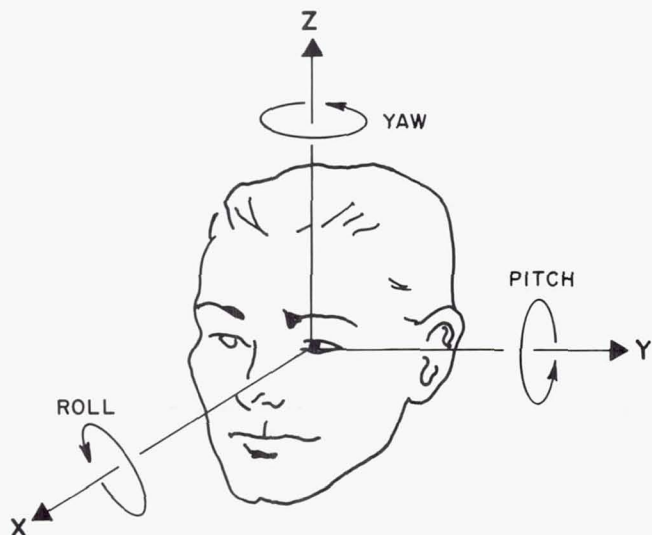


FIG. 1. Definition of axes for linear and angular motion. [From Hixon et al. (89).]

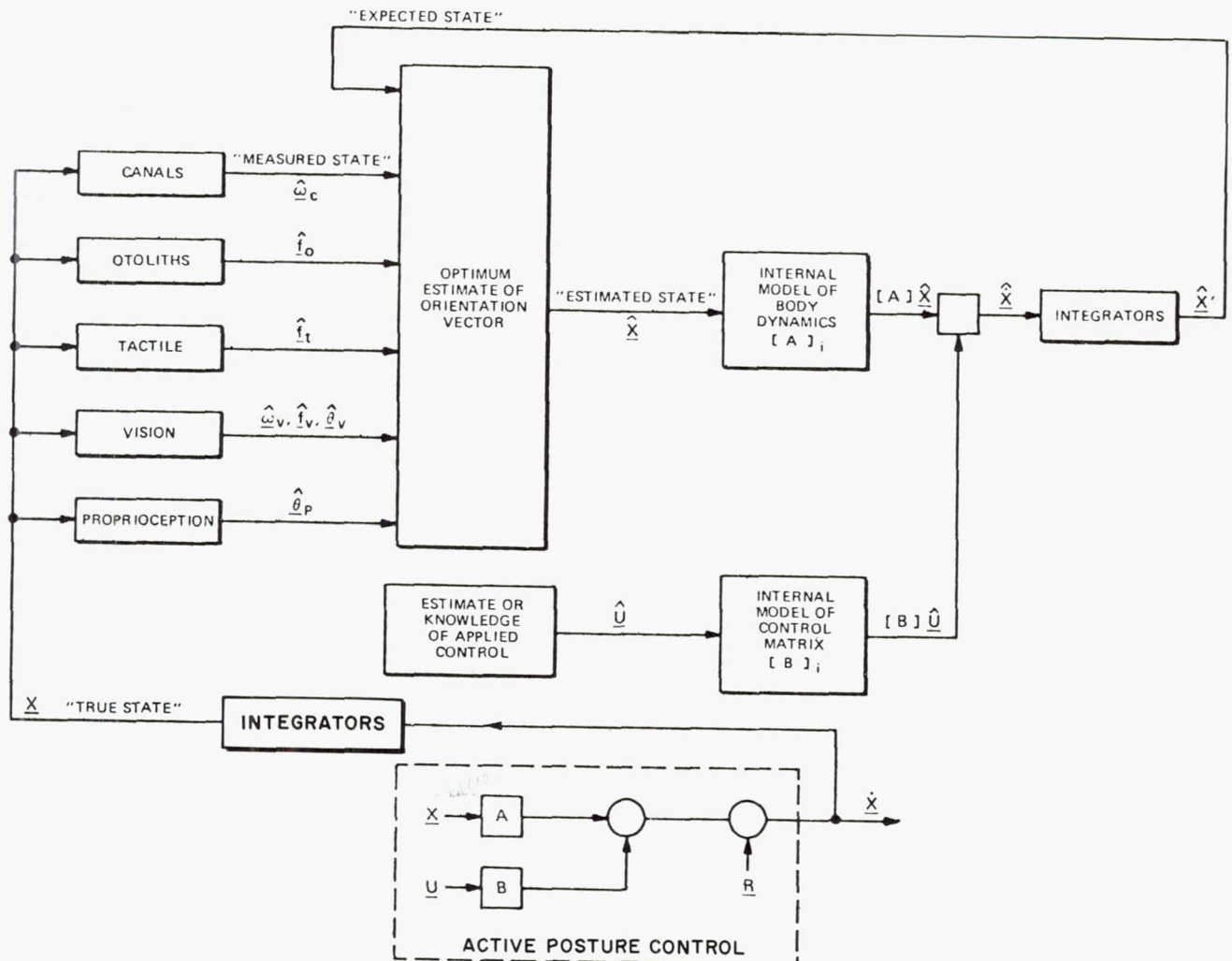


FIG. 2. Schematic representation of spatial orientation process. "True state" vector, \underline{X} , consisting of linear and angular positions and velocities, is produced by changes resulting from three sources: unforced behavior of body, $\underline{\dot{X}}$ processed by A, commanded body changes, commands \underline{U} processed by B, and unmeasured disturbances, \underline{R} . Various sensors are each responsive, especially to one or more components of measured state. Symbol " $\hat{\cdot}$ " indicates an estimate of the vector; ω , angular velocity; \underline{f} , specific force, gravity minus linear acceleration; θ , angle. Measured state signals are combined with "expected state," $\hat{\underline{X}}$, derived from a presumed internal model of the body, in optimum estimator to produce estimate of orientation, $\hat{\underline{X}}$. [From Young (174).]

ducers only for stimulation frequencies so low (less than 0.1 Hz) as to lie in the range of man-made transportation and amusement devices rather than in the normal physiological range.

Otolith Organs

The otolith organs, discussed more fully in the chapter by Goldberg and Fernández in this *Handbook*, are linear accelerometers and, as such, respond to linear accelerations and to changes in orientation with respect to the gravity vector. The otolithic membrane in each utricle and saccule, made more dense than the surrounding endolymph fluid by the presence of numerous calcite crystals, slides downhill when the head is tilted and lags behind when the head is accelerated with respect to inertial space. Between the

utricle and saccule these organs represent acceleration sensitivity in all three dimensions. They are the principal nonvisual determinants of static orientation with respect to the vertical. In conjunction with the vertical semicircular canals they also act to signal changes in orientation and to initiate corrective postural responses. The implied ambiguity in the use of otolith information for static orientation (physical devices cannot distinguish between linear acceleration and gravity) is normally solved by interpreting the otolith signals on the basis of other sensory information, including semicircular canal afferents.

Somatosensory Cues

Although the primary nonvisual orientation cues in humans are vestibular in origin, the influence of non-

vestibular signals must not be ignored. The classic nonvestibular righting reflexes seen in labyrinthectomized animals, in response to asymmetric tactile cues, are dramatic examples (156). In humans the ability of labyrinthine-defective subjects to retain orientation and balance, even in the dark, is ample indication of the potential utility of nonvestibular orientation cues. For those rare situations in which vestibular cues are altered temporarily, such as in the weightless condition of space flight, tactile and proprioceptive cues appear to substitute for some aspect of otolith signals. The proprioceptive and tactile cues are of essentially three kinds: pressure cues, limb position signals, and muscle length and tension afferents.

The quantitative relationships between sensory characteristics of surface and deep pressure somatic endings and perception has received relatively little attention. The high-frequency pass band of many of the pressure endings corresponds to perception of vibration rather than phase-dependent orientation oscillations. There is, however, a remarkable similarity between the sensitivity curves of various somatosensory endings as a function of vibration intensity and frequency and the corresponding psychophysical curves for detection of local vibration.

Pacinian corpuscles are deep pressure sensors with a rather distinctive morphology. They are encapsulated by cylindrical fluid-filled sheaths, or lamellae, that prevent static deformation from reaching the hard elliptical core. It is this dendritic core that is actually responsible for producing generator current, and thus the capsule acts as a mechanical high-pass filter of deformation stimuli. Because of the mechanical structure of the capsule, when a pressure is released the core tends to distend along the orthogonal axes and also to produce a generator current. Pacinian corpuscles show no significant static response to compression stimuli and are quickly adapting, displaying a time constant of 1–10 ms.

Being deep pressure sensors, Pacinian corpuscles have a fairly wide receptive field and respond to stimuli not directly above the corpuscle. They are exquisitely sensitive and respond to displacements as small as 10 μm (105).

There are also two types of pressure sensors located close to the surface of the skin. Type I receptors are formed by myelinated fibers that end in Merkel cells near the surface of the skin. Merkel cells are found within domelike elevations of the epidermis between hair follicles known as Iggo corpuscles. Type I receptors exhibit highly focused receptive fields and respond only to direct stimulation of the touch corpuscle. They respond dynamically to stimuli as small as 1–5 mm of skin displacement. The step response adapts with time constants of about 1 s and about 30 s. Type I receptors show a static response, but it is characterized by a highly irregular afferent rate. These fibers usually do not exhibit any resting discharge (see ref. 92).

Type II receptors are formed by myelinated fibers ending in lightly encapsulated Ruffini endings. The

end organ is situated in the dermis but is not as close to the skin as the type I receptors. Their linear transfer function to skin displacement can be fit with three adaptation time constants of approximately 1, 5, and 20 s. Type II receptors exhibit a regular static response as well as a regular resting discharge and have a relatively wide receptive field responsive to stretch (see ref. 29).

Psychophysical studies of human threshold to skin vibration suggests two receptor populations, one population being sensitive to very high frequencies. These results are consistent with quickly adapting Pacinian corpuscles beneath the dermis and more slowly adapting cutaneous receptors, as described in neurophysiological studies.

Psychophysical studies have also shown that the threshold tends to decrease with increasing stimulus area (107, 161). The principal function of somatosensory information in the normal system is apparently for rapid detection of changes in surface force, which corresponds to changes in acceleration under most circumstances (122).

Limb Position

Body-orientation perception also depends heavily on the perceived orientation of the various joint angles. Not only are limb-position angles relevant to postural control, but the orientation of the head with respect to the trunk is especially critical. Because vestibular and visual cues are measured in a head-fixed coordinate system and yet the postural reactions to the appropriate muscles must be in a different coordinate system, it is obvious that the relative orientation of the head, trunk, and limbs must be known reasonably accurately. The common notion that these joint angles are sensed exclusively or even primarily through the joint receptors in the joint capsules has been called into question. Although gross and monotonic signals relating joint angle to capsule sensor afferents certainly exist, they may not account for the accurate perception of limb position that is clearly at work in many human tasks. Among other considerations, these capsule receptors, which are really pressure receptors, are influenced by total force across the limb, which in turn is subject to external variables.

The muscle receptors, muscle spindle afferents, and Golgi tendon organs, once believed to play no role in proprioception or kinesthesia, are now generally acknowledged to be of importance in determining limb position and human spatial orientation. Muscle spindle afferents of several types signal the length of intrafusal muscle fibers, which in turn relate to overall muscle length and its rate of change, as well as to the intended or regulated muscle length commanded by the γ -motor-control signals. Golgi tendon organs are capable of monitoring total force in a portion of a muscle and therefore indirectly of monitoring the weight of the head or a limb. Both spindle and tendon organs can therefore play two roles relative to human

spatial orientation. As force sensors they can serve as additional linear accelerometers in which the force required to maintain head or limb position is a measure of both orientation with respect to gravity and net linear acceleration. One of the cues to a pilot that he is in a high-acceleration turn, for example, is that his head feels heavy and that it is an effort to maintain his arm position. Additionally, in the nonaccelerating environment these signals can augment the joint capsule receptors in signaling the orientation of the head with respect to the trunk. The effect of artificial spindle stimulation on perception of limb orientation is easily demonstrated in humans by the ability of an externally imposed vibration over a muscle to produce the perception of muscle shortening (65). Supporting physiological evidence comes from the discovery of cortical representations of certain muscle spindle afferents (59). Specific application of vibration to certain postural muscles can produce predictable changes in body orientation. The strength of body-orientation illusions in response to this artificial spindle stimulation is variable among subjects (101).

PSYCHOPHYSICAL MEASURES OF PERCEPTION OF ORIENTATION AND MOTION

Throughout this chapter, reference is made to experimental results about subjective orientation, or the perception of motion. Obviously we have no means of measuring what the subject thinks or feels except by his own indications. (Electrical or magnetic evoked responses are not current useful alternatives for this purpose.) Two general categories of quantitative indications of orientation and motion perception are used. The first of these makes use of magnitude estimation either as a concurrent or retrospective judgment. The notion of attaching quantitative estimates to sensation magnitude and relating them in turn to objective physical stimuli builds on the early work of Weber (166a), modified by Fechner (48a), leading to the logarithmic sensitivity notion that a just noticeable difference (JND) in stimulus is proportional to the stimulus level. In performing magnitude estimations, quantitative subjective estimates of a scalar quantity are made in relation to a previously learned or revealed reference scale. The techniques of appropriate magnitude estimation methodology are reviewed by Stevens (149, 150) and by Poulton (134). These techniques include pinning down the end and the midpoint of the scale, refreshing the subject with calibration exposures to the known stimuli, and avoiding placing test stimuli close to the ends of the reference scale. Ratio estimates are particularly useful. When the estimate is made during the stimulus, it is known as a concurrent estimate. However, the subject may also wait until the stimulus is completed to judge how fast he was moving or how far he had moved (integrating the subjective velocity judgment), in which case it may be referred to as a retrospective estimate. Guedry et al. (83) have

demonstrated how concurrent and retrospective estimates can lead to very different magnitude estimates, using the situation of triangular velocity wave forms for rotation about a vertical axis as an example. Retrospective subjective angular displacement judgment yields total displacement estimates reasonably close to true angular displacements and is predicted well by the torsion pendulum model referred to in the chapter by Goldberg and Fernández in this *Handbook*. When the same type of displacement estimate is made continuously, concurrent with the stimulus, and the subject attempts to always keep the pointer stationary in space, the total displacement is substantially less, as indicated in Figure 3. This possible influence of the concurrent magnitude estimation task on the perception being measured is not often considered, but it may be of some significance in explaining certain perception results. A task of magnitude estimation need not involve an arbitrary scale or even practice on the reference scale. For example, many of the results on tilt perception, including the rod and frame test and the tilted-room experiments (170), rely on experiments in which the subject is required to align a visually observed line or a calibrated rod to the perceived vertical, and the angle between this alignment and the true vertical is taken as the error in the perceived tilt. No extensive instruction on the meaning of the vertical as a reference scale is required.

An alternate method of quantifying perception is a response-nulling technique. In the nulling method, the subject actively controls his own stimulus in order to return himself to a subjective reference level. For example, to assess the magnitude of visually induced tilt the subject may be required to actively orient

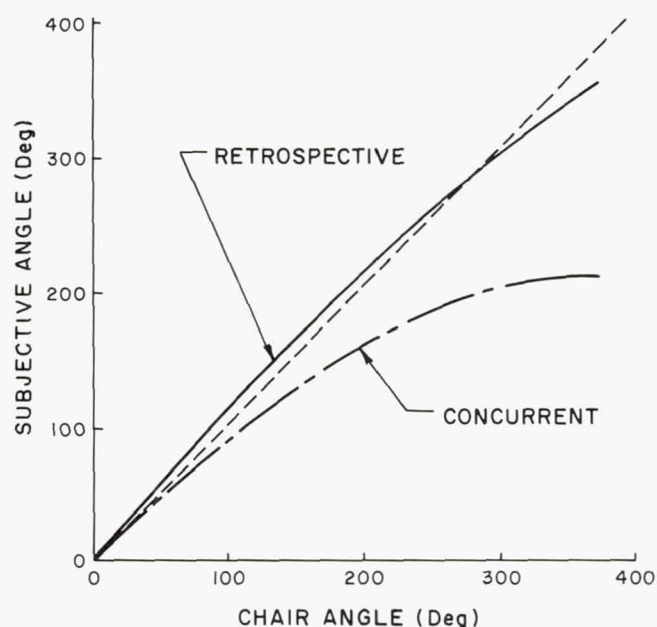


FIG. 3. Subjective estimates of angular displacement (produced by triangular velocity wave form) are greater for retrospective than for concurrent estimates. [Adapted from Guedry (76).]

himself to a posture that he perceives to be upright. Alternatively, the subject might control the angular velocity of a visual field or his own actual inertial rotation in the presence of some test stimulus until he feels that he is stationary. In a final variation, that of sequential matching, the subject attempts to give a quantitative indication of his perception of motion or tilt, induced by one stimulus, by subsequently matching the sensation through another stimulus.

The nulling method and the sequential matching method share the advantage of avoiding substantial confusion about the subject's interpretation of which physical variable is to be estimated.

ANGULAR ACCELERATION

Rotation in Dark

The most frequently studied motion sensation results from rotation about a vertical axis in the dark. The dizziness that comes from opening one's eyes during constant-velocity turning or after having been brought to an abrupt stop is a common childhood experience. It is easily explainable on the basis of stimulation of the semicircular canals in a single plane. When the head is pitched approximately 25° forward from the erect position the human horizontal semicircular canals lie approximately in the horizontal plane, and rotation about a vertical axis stimulates primarily but not exclusively these semicircular canals. The corresponding semicircular canals are neither precisely coplanar nor very close to orthogonality with the other canals in each labyrinth (12). When the axis lies through the head, centripetal acceleration effects on the otoliths may be neglected. The simplicity of this stimulus axis has made it particularly attractive for use in indirect measurements of semicircular canal

mechanics. The earliest of these attempts at "cupulometry" was accomplished with steps of angular velocity. The torsion-pendulum model, discussed in *Torsion-Pendulum Model*, p. 1030, would predict a rapid rise and a slow exponential decay to null following a step in angular velocity, but the actual experience is somewhat different. Neither subjective measurements nor nystagmus can reveal the short time constant of fractions of a second. The subjective response does, however, decay right through zero to yield a period of reversed sensation. Subjective response to a long-duration constant velocity step, followed by a sudden return to zero angular velocity, is shown in Figure 4. The initial decline in subjective velocity is nearly exponential. The similarity between this response and the predicted return of the cupula according to the torsion pendulum equation led to the use of subjective sensation decline, as well as of nystagmus decline, following velocity steps as a means of estimating the long time constant, Π/Δ —the ratio between viscous and elastic coefficients. The differing slopes of the response declines for subjective sensation and for nystagmus, however, indicate the involvement of more than cupula dynamics alone (72).

Models for vestibular adaptation to horizontal rotation allow for the possibilities that cupula responses are not mirrored exactly in either nystagmus or subjective sensation. (Recent developments in single-unit recording of first-order afferents in some species and of recordings from the vestibular nucleus and thalamus of other species of monkeys lead to the current view that the time course of cupula response, which may be as short as 5–7 s in the monkey, is lengthened to give a considerably longer response time at the level of the vestibular nucleus (21)). This longer time constant may be approximately 16–20 s and reflects rather accurately the time constant of decay of vestibular

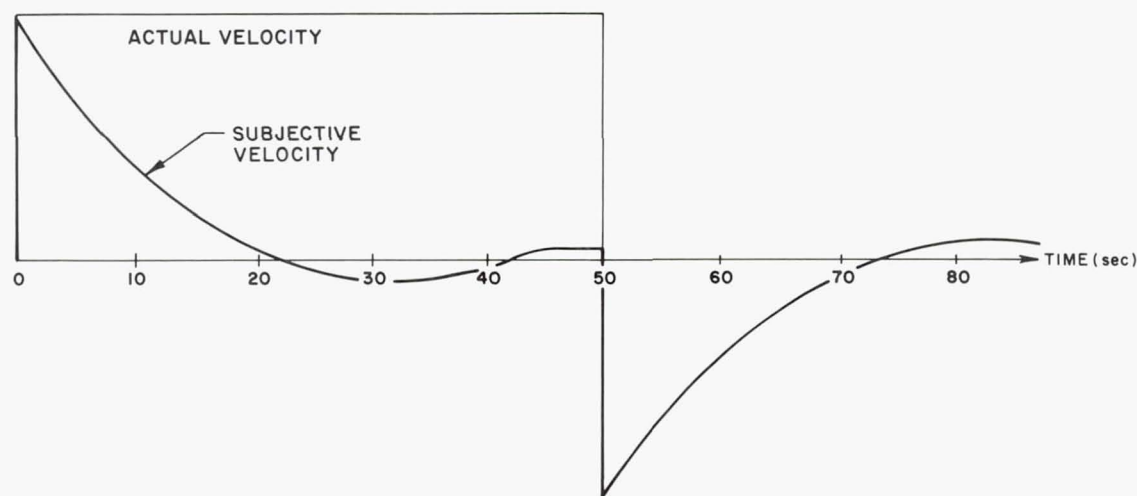


FIG. 4. Subjective angular velocity decays and may reverse during prolonged constant-velocity rotation. Sudden stop after prolonged rotation elicits transient, oppositely directed, postrotatory response.

nystagmus. Subjective sensation in man is known to decrease with a shorter time constant than that of vestibular nystagmus.

Yet another difference in the dynamic responses observed from afferent records from those seen in nystagmus recordings or in perception responses occurs in rotation about other than the z -axis. Examination of the time course of postrotatory sensation or nystagmus for rotations about each of the three principal body axes (x , y , and z), always about a vertical axis, reveals a significant difference between axes, provided the stimulus is of long enough duration (7, 34, 76, 82, 112, 114). The long time constant for rotations about the body pitch axis and roll axis for a vertical axis are approximately one-half as long as the time constants for rotation about the body yaw axis. Investigations of the outputs of the individual semicircular canals in squirrel monkeys, however, reveal no such differences in time constants between axes (49). Once again it appears that higher center processing alters the effective time constant from that received from the afferent signal. Certainly the movements normally encountered in pitch and roll, even about a vertical axis, are of briefer duration than for yaw. An integration time constant of more than a few seconds would not be required to reproduce the pitch and roll transient movement faithfully.

Active Versus Passive Movement

The preceding discussion of postrotatory sensation is applicable strictly to the case where a subject is passively rotated and stopped. Under conditions of active rotation, knowledge of the rotation situation and information supporting the perception of continued rotation at constant velocity may change the perception. In one extreme example, it has been demonstrated that subjects who maintain the sustained angular rotation about a vertical axis by active movements and then are suddenly decelerated generally show postrotatory sensations in the same direction as the original sensation, even though their postrotatory nystagmus reversed direction, just as for passive rotation (39, 80). Sensory responses due to active movements are interpreted differently from those due to passive movements. One simple method of thinking of this effect is that active movements provide another and very powerful measurement, consistent with the "efferent copy" concept of von Holst and Mittelstaedt (162) and of others. When the subject "knows what movement was intended" on the basis of his active control, sensory information and particularly conflicting sensory information may be reinterpreted or discarded when inappropriate.

Torsion-Pendulum Model

The angular-velocity perception resulting from stimulation of a pair of parallel semicircular canals can be related to the mechanics of semicircular canal

transduction, to the afferent firing rate of primary vestibular neurons, and to central neuronal activity. Functional interpretation of Steinhausen's (148) observations and subsequent formalization by van Egmond et al. (160) of semicircular canal function in terms of a torsion-pendulum model was based on correlation of the physical model with the time course of subjective perception for rotation of the subject about a vertical axis. Subsequent investigations, especially the direct measurement of first-order afferent signals (49) and central vestibular neurons (115), have revised the earlier view that canal afferents directly drive the slow phase of nystagmus or the subjective angular velocity. Nevertheless, the concept of a relationship between the time course of cupula deflection and the subjective sensation remains a valuable one.

The essence of the torsional pendulum model is spelled out in the chapter by Goldberg and Fernández in this *Handbook*. To recall, endolymph displacement, ξ , is related to head acceleration (with respect to inertial space) in the plane of the canal by the equation

$$\frac{\Theta d^2\xi}{dt^2} + \frac{\Pi d\xi}{dt} + \Delta\xi = \Theta\alpha(t) \quad (1)$$

where Θ is the moment of inertia of the endolymph ring, including the fluid in the ampulla and utricular sac; Π is the viscous drag coefficient; Δ is the spring constant; and α is the angular acceleration of the head. Because of the high ratio of viscous damping of endolymph flow in the semicircular canal Π to the moment of inertia of the ring of endolymph Θ the inertial reaction torque on the ring of fluid attributable to its acceleration with respect to inertial space, $\Theta[(d^2\xi/dt^2) - \alpha(t)]$, is very quickly balanced by the viscous damping torque, $\Pi d\xi/dt$. The time constant associated with this process is the short time constant, $\tau_s = \Theta/\Pi$, referred to in the chapter by Goldberg and Fernández in this *Handbook*, with a value on the order of 3–5 ms. Within three time constants (9–15 ms) the torque balance is 99% complete, and the endolymph flow, $d\xi/dt$, is proportional to the head acceleration $\alpha(t)$. Consequently endolymph displacement ξ is then proportional to the head angular velocity, $\int \alpha(t) dt$. To the extent that the change in firing rate of canal afferents reflects endolymph displacement, or more properly shear force across the subcupula space of the crista, the afferents signal head velocity for head motion durations much longer than τ_2 and shorter than τ_1 . The long time constant of the semicircular canals, $\tau_L = \Pi/\Delta \approx 5$ to 15 s, is attributable to the relatively weak elastic-restoring torques of the cupula and membranous canal that oppose any deflection of endolymph by a spring torque $\Delta\xi$. The weak restoring forces must oppose the substantial damping $\Pi d\xi/dt$ in returning the cupula and the hair cells to their original position. For acceleration stimulus periods comparable to or longer than τ_L , however, these restoring forces are significant. Consequently for a step of angular acceleration after the first few milliseconds the theo-

retical endolymph displacement increases according to the equation

$$\xi_1(t) = (\Theta/\Delta)\alpha[1 - \exp(-t/\tau_L)] \quad (2)$$

as discussed in the chapter by Goldberg and Fernández in this *Handbook*. If the torsion-pendulum model entirely explained the dynamics of peripheral neurons, central vestibular neurons, and perception of velocity, the perceived angular velocity following the onset of a sustained constant acceleration in the dark would resemble the exponential equation (Eq. 2). In fact, the actual time course of subjective velocity about a vertical axis, as shown in Figure 5, departs from a torsion pendulum only after approximately 30 s. For brief accelerations (0.6–6 s), the perceived velocity is roughly proportional to the true velocity of the head. For such motions, which cover the physiological range of head motion (0.1–1.0 Hz), the integrating property of the endolymph viscous damping makes the semicircular canals resemble integrating accelerometers, and they yield accurate velocity estimates in daily activity. [Absolute measures of subjective velocity are difficult to obtain. Authors frequently report the indicated velocity to be up to 50% greater than the actual velocity during the first 15 s of exposure (74, 78).] Of course these velocity estimates must be integrated

once more by the central nervous system to produce estimates of angular orientation—even as used in the “90-degree turning points” method of subjective rotation-magnitude estimation. Errors in this second integration, including scaling, loss of initial conditions, and imperfect integration, lead to methodological problems in judgment of azimuth orientations in the laboratory (see, for example, ref. 81).

For sustained accelerations of medium duration (6–25 s), the perceived velocity gradually drops below the linearly increasing head velocity and approaches an asymptotic constant velocity proportional to the imposed acceleration, with a time constant of 12–15 s. The rise to a constant level during a constant acceleration is basically similar to the step response of the torsion-pendulum model and the nonadapting semicircular canal afferents (see Fig. 9 in the chapter by Goldberg and Fernández in this *Handbook*). It would be a mistake, however, to assume that cupula pressure or displacement is directly encoded to yield subjective velocity. First of all, the time constant of the rise (12–15 s) is probably longer than human horizontal canal response, based only on dimensional analysis [(38, 116); I. S. Curthoys and C. M. Oman, unpublished observations] and on the known squirrel monkey canal long time constant of approximately 5 s. Second, com-

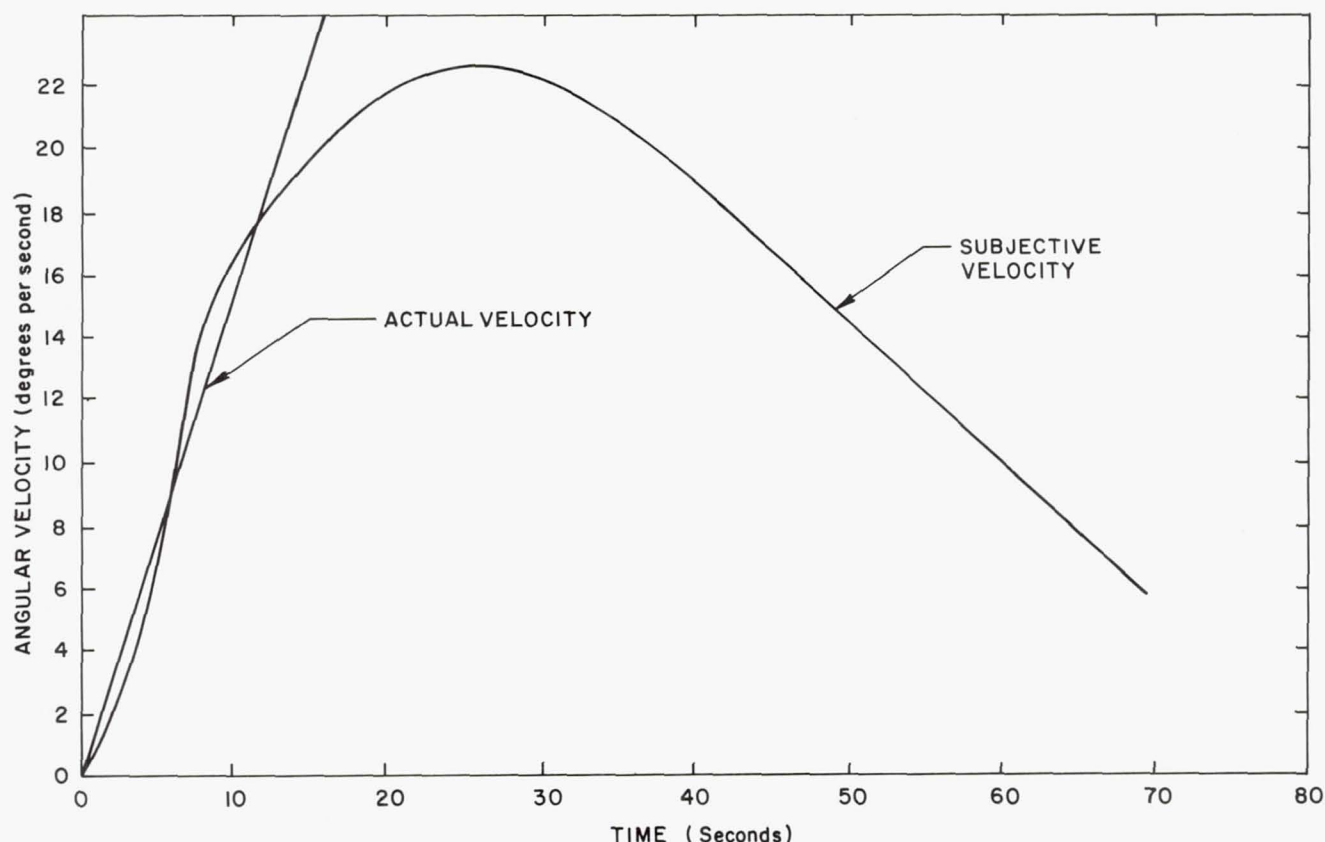


FIG. 5. Subjective velocity follows actual velocity only for 1st 10 s of constant angular acceleration, then plateaus and actually decays. [Adapted from Guedry and Lauver (79a).]

missural pathways from the contralateral labyrinth (inhibitory with delay) as well as vestibular efferent signals (partially disrupted by anesthesia) probably lengthen the apparent time constant to approximately equal the value of nearly 20 s seen in the slow phase of vestibular nystagmus. One cannot yet eliminate the possibility that those (few) fibers that go directly from the eighth nerve to the cerebellum without first synapsing in the vestibular nucleus are responsible for rotation perception, thereby invalidating the above argument. Recordings of unit activity in the monkey thalamus (24, 25) also show an effective time constant for responses to angular acceleration which, although longer than the canal afferents, is shorter than the time constant associated with nystagmus.

Thresholds

To put into perspective the various experimental reports of acceleration-sensation threshold, time to detect, and duration of sensation, it is useful to return once more to the predicted cupula deflection following from the torsion-pendulum theory (76).

According to the simplified threshold notion of minimum detectable sensation related to threshold cupula position or pressure, the responses to acceleration and velocity steps are implicit in the simple exponential wave forms of Figure 6A (acceleration step) and Figure 6B (velocity step). For an acceleration step, the time course of calculated cupula deflection is

$$x(t) = \alpha \tau_S \tau_L [1 - \exp(-t/\tau_L)] \quad (3)$$

and the time required to reach a given threshold level (x_{\min}) is given by

$$t_{\text{detect}} = \tau_L \ln[\tau_S \tau_L \alpha / (\tau_S \tau_L \alpha - x_{\min})] \quad (4)$$

Clearly, for $\alpha \leq x_{\min}/\tau_S \tau_L$ the time to detect becomes infinite, thus defining a threshold acceleration α_{\min} . In fact, the curve shown in Figure 7 matches the formulation extremely well. Note further that for all but the lowest (near threshold) accelerations, the curve is close to the hyperbola, $\alpha t_{\text{detect}} = C$ (a constant). Another way of stating this approximation is: for stimulus durations that are short relative to τ_L , the cupula deflection or pressure rises almost linearly with time following an acceleration step, and so the threshold

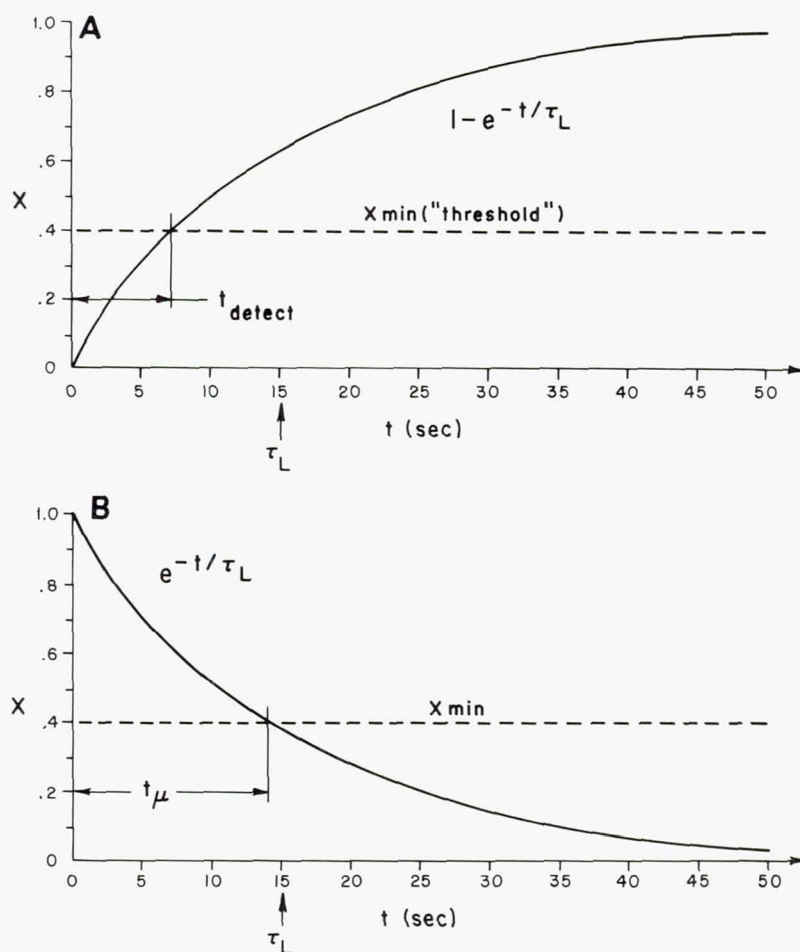


FIG. 6. Normalized torsion-pendulum response, X , for system with long time constant, $\tau_L = 15$ s. A: rising response to acceleration step is detected when it reaches X_{\min} . B: decaying response following velocity step has duration t_μ .

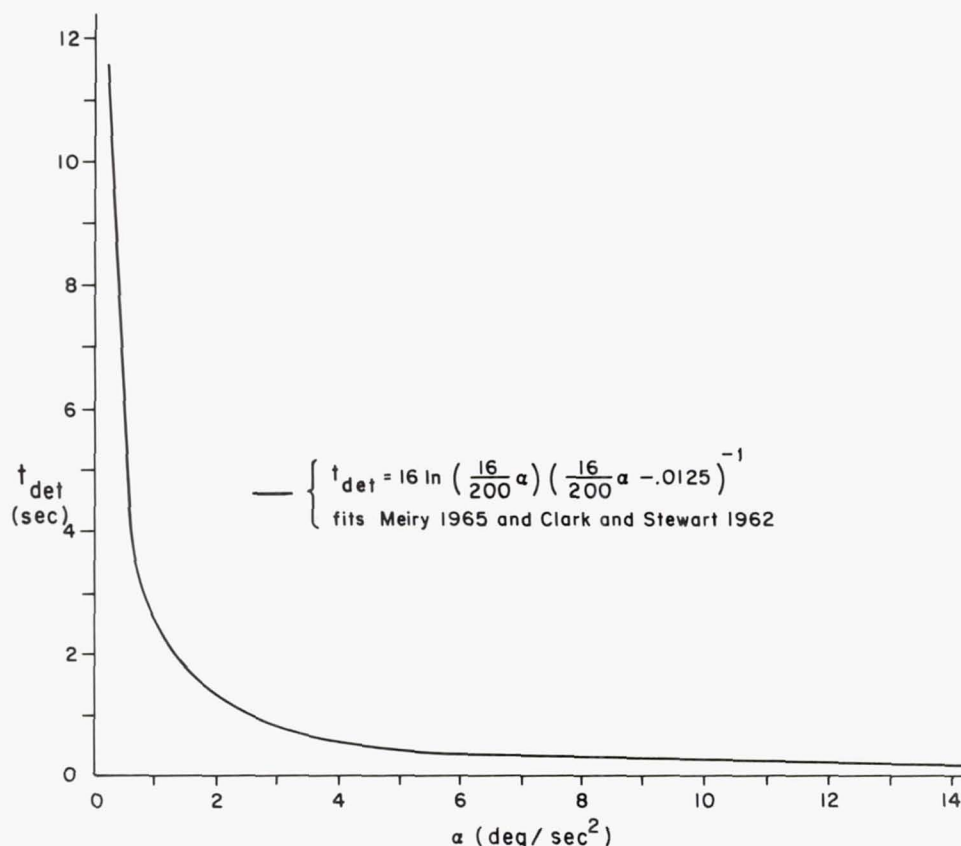


FIG. 7. Time to detect, t_{det} , step of yaw angular acceleration of magnitude α increases sharply for acceleration levels less than 2–3 deg/s^2 . [Adapted from Guedry (76).]

level is reached when a certain critical velocity is attained, independent of the acceleration amplitude. This critical velocity, known as the Mulder product, is merely the minimum detectable angular velocity for brief steps of acceleration

$$\omega_{min} = \alpha_{min} \tau_L$$

or $\omega_{min} \approx 2.5 \text{ deg/s}$, assuming $\tau_L = 10 \text{ s}$, $\alpha_{min} = 0.25 \text{ deg/s}^2$, or $\tau_L = 12.5 \text{ s}$, $\alpha_{min} = 0.20 \text{ deg/s}^2$. For lower accelerations, of the order of only two or three times threshold (less than 1 deg/s^2), even the theoretical cupula deflection departs significantly from linear growth long before a threshold is crossed. Consequently, for small accelerations t_{detect} is longer than predicted by the Mulder product and is given by Equation 4.

Another use of the concept of a cupula-deflection threshold is in interpreting postrotatory motion sensation or nystagmus. As indicated in Figure 6B, the calculated cupula deflection following a sudden change in angular velocity, such as a stop after sustained constant velocity rotation of 1 min, is a rapid peak followed by an exponential decay to zero with time constant τ_L

$$x(t) = \omega \tau_S \exp(-t/\tau_L) \quad (5)$$

where ω is the size of the angular velocity step. On the

assumption of a cupula-deflection threshold x_{min} , the sensation of rotation has a duration t_μ (76) found from the equation

$$x_{min} = \omega \tau_S \exp(-t_\mu/\tau_L)$$

to yield

$$t_\mu = \tau_L [\ln \omega + \ln(\tau_S/x_{min})] \quad (6)$$

The first term of Equation 6 indicates that the duration of sensation would be proportional to the log of the velocity step and that the constant of proportionality (slope) would be the long-time constant τ_L . Such plots of sensation duration versus stimulus velocity for sudden stops, called sensation cupulograms, are roughly straight lines when plotted on semilog paper and were used in early estimates of τ_L , yielding values in the region of 10–15 s (28, 160).

The concept of a threshold value of a physical stimulus for its detection seems simple enough: merely the minimum level of that stimulus or the minimum change that can be reliably and immediately detected. In practice, the notion of threshold for detection of angular acceleration is not nearly as simple. Several problems are associated with the estimate of τ_L from a sensation cupulogram or even from continuous magnitude estimates of velocity. First of all the decay is not really a simple exponential but exhibits adaptation

and overshoot, as discussed in *Adaptation*, p. 1035. Therefore, any estimation of a single time constant will be too small if the adaptation effect is neglected. Second, the slope is less steep for the nystagmus cupulogram than for sensation, which is not consistent with a simple interpretation of cupula deflection totally explaining the dynamic response of both sensation and nystagmus. Third, the phenomenon of habituation or reduced response to repeated stimulation can easily lead to a sensation cupulogram with a slope that is too small (τ_L too low) if tested with successively increasing velocities or too large if tested with decreasing velocities (28). Threshold or duration measures for nystagmus are typically based on the presence of a fast phase, or beat, rather than the associated slow compensatory eye deviations. The first ocular reactions to low semicircular canal signals are these slow eye deviations, however, and nystagmus represents an overload condition of sorts. Beyond these methodological problems lie some fundamental limitations in considering a hard threshold x_{\min} associated with a minimum cupula deflection. No such threshold has been reported in either individual first-order afferent recordings or unit recordings from neurons in the vestibular nuclei (see the chapter by Goldberg and Fernández in this *Handbook*.)

Sensation measures always depend on the subjective willingness to risk an opinion based on minimal information. If the response is to be the subject's reporting of a detectable motion, care must be taken to control such variables as the subject's willingness

to guess or a tendency for the subject to wait until he is certain before hazarding an opinion. Normally, distinguishing the inherent detectability of a signal from the subject's strategy is accomplished by signal detection theory and by use of receiver operating characteristic curves. In one common implementation, the subject was forced to choose one direction or another for a test at a fixed time after the test was initiated. By convention, the acceleration magnitude that leads to 75% correct detection is associated with the threshold. For the double-staircase method of threshold determination, the subject is presented with two staircases of stimulus levels, one initially much higher and one much lower than the presumed threshold. After each correct identification, the following stimulus from that staircase is lowered by a fixed ratio and after each error the subsequent stimulus presentation is raised. In this manner the upper staircase descends and the lower staircase ascends toward the threshold level. The two staircases finally cross and recross, yielding an estimate of the actual threshold.

The entire concept of a vestibular rotation threshold is better couched in terms of information or signal detection than in terms of a physical switch. Consider, for example, a combined stimulus pattern, c , consisting of a simultaneous threshold velocity v_t and threshold acceleration step a_t in the same direction (see Fig. 8). The calculated cupula deflection never exceeds the hypothetical hard threshold level x_{\min} for any of the three stimuli. If the simple concept of a cupula threshold were adequate, the combined stimulus would be

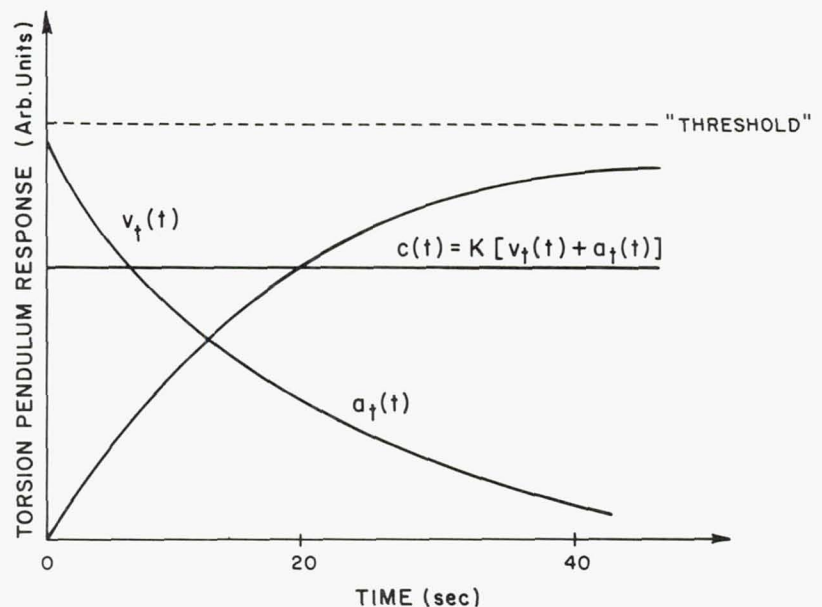


FIG. 8. Theoretical response of the torsion-pendulum cupula model to threshold velocity step, $v_t(t) = e^{-t/18}$, to threshold acceleration step, $a_t(t) = 1 - e^{-t/18}$, and to a combined stimulus $c(t) = K[v_t(t) + a_t(t)]$. In theory, calculated cupula response for $c(t)$ never exceeds threshold response to velocity or acceleration threshold steps alone and would be undetectable for K as large as unity. Measured thresholds for $c(t)$ tended to values of K below 0.75, lending support to signal-detection model rather than hard limit model for threshold. [Adapted from Ormsby (131).]

just barely detectable or still at threshold when it was the sum of v_t and a_t . Experiments concerning the detectability of various combinations of acceleration impulses v_1 and steps a_1 showed that detection thresholds were always lower than predicted on the basis of cupula deflection alone (131). Not only the peak cupula deflection but the duration of its deflection contributed to stimulus detectability, and they support the concept of threshold in terms of a signal-detection process. Recall that the resting discharge rates of both regular and irregular first-order afferents vary from moment to moment in the absence of any stimuli. The signal-detection model assumes that an estimator must identify a probable signal from a noisy process consisting of the parallel firings of many afferents. For signals that are small compared to the noise standard deviation, a longer processing time is required (131). This signal-in-noise theory is consistent with the psychophysical threshold and detection-time measures, and with the absence of any clear threshold in afferent rates. Furthermore it is easily extended to explain experiments in which the detection time is biased by the expected direction or time of an acceleration or by visually induced motion (176).

Oculogyral Illusion

Thresholds for detecting motion in the presence of a light are one-half to one-tenth the size of those measured in the dark (31, 68, 74, 91, 136, 159). When the subject is rotated in the dark and has a single light source fixed in front of him, that light source also appears to move relative to the observer. This illusion of movement is referred to as the oculogyral illusion. It was initially explained on the basis of noncompensated slow-phase eye movements associated with vestibular nystagmus. These slow movements in the direction opposite to the acceleration stimulus would produce retinal slip of the observer-fixed target in the same direction as true-target motion relative to the observer in the direction of his acceleration. It was presumed that suppression during fast phases was present. However, a visual target normally inhibits or suppresses vestibular nystagmus, at least below the resolution of most eye-movement measurements, and so the simple eye-movement explanation does not hold. The oculogyral illusion is seen even with after-images, which permit no retinal slip (173), although Byford (26) reported its absence under stabilized image conditions. The oculogyral illusion may still be caused by attempted eye movements if the process of voluntary visual suppression of vestibular nystagmus involves a reinterpretation of the position of a target fixed in the visual field (168). Although an adequate explanation for the oculogyral illusion is still not at hand, it is generally assumed that it results from a vestibular signal. The labyrinthine information indicates that not only is the observer moving but also that everything fixed relative to him is also in motion

in the same direction, including touched objects and sound sources. The part that remains unclear is why the light should appear to move even farther relative to the observer. Although it is impossible to eliminate the argument that the task of observing the light raises alertness levels and consequently lowers the measured level, it appears that the perceived movement of the light itself contributes to this lower threshold (91).

Adaptation

Although the torsion-pendulum model for mechanical events in the semicircular canal is useful for explaining the short-term mechanical events and for describing in a general sense the dynamic response of perception and nystagmus to brief acceleration stimuli, this model is clearly inadequate for responses that last more than 20–30 s. For example, the torsion-pendulum model predicts that the steady response to a sustained constant acceleration is going to be a constant cupula deflection. The observed subjective velocity, however, reaches a peak at 25 s after the onset of the constant acceleration and then proceeds to plateau and decrease slowly back to zero, as indicated in Figure 5. Slow-phase nystagmus velocity shows a similar behavior, but significantly it does not peak until 80–100 s after the initiation of the acceleration (108). Unless a more complicated mechanical mechanism is accepted for cupula creep back toward its initial conditions, such as underdamped cupula dynamics (27) or sliding of the cupula over the crista (111), or for efferent signals stiffening of the cupula, some manner of neural adaptation must be assumed to explain this behavior. The adaptation seen in long-duration responses affects neither the calculations of perceived angular velocity during brief acceleration nor the time to detect any but the lowest accelerations.

Another major difference between the torsion-pendulum predictions and behavioral measures seen is illustrated in the long-duration response to an acceleration impulse or a step change of angular velocity, as shown in Figure 4. The simple torsion-pendulum model predicts a single exponential decay from a peak value back toward zero, with a time constant of τ_L . Both subjective angular velocity and slow-phase nystagmus velocity not only decay back toward zero but also overshoot, resulting in a secondary phase of post-rotatory sensation or secondary nystagmus. Once again the time at which the secondary phase occurs is much earlier for sensation (approximately 30 s) than for nystagmus, in which case it may not appear for more than a minute. This evidence of adaptation is consistent with the observations from cupulograms, discussed above in this subsection, that the slope of the cupulogram is different for subjective velocity than for nystagmus, leading to the false conclusion of a shorter semicircular canal time constant τ_L based on sensation. An overall black box model, which appears adequate to describe the dynamic relationship among

angular acceleration, subjective velocity, and nystagmus slow-phase velocity, is given by the transfer function (178)

$$\frac{\hat{\omega}(s)}{\omega_i(s)} = \frac{Ks^2 e^{-\tau_d s}}{(\tau_S s + 1)(\tau_L s + 1)(\tau_a s + 1)} \quad (7)$$

where $\hat{\omega}$ is subjective estimate of angular velocity; ω_i is actual inertial velocity of head; K is proportionality constant; τ_d is pure delay time (0.3 s for subjective sensation); τ_S is short time constant (on the order of 0.005 s); τ_L is long time constant (16 s); τ_a is adaptation time constant (30 s for subjective sensation, 120 s for nystagmus); s is the generalized complex variable in Laplace transform notation. Slightly different formulations leading to similar models were independently developed by Malcolm and Melvill-Jones (108) and were discussed in a conceptual manner earlier by others (73, 79).

The site of adaptation is not entirely clear. It probably does not take place mechanically in the semicircular canal itself, because only some of the first-order afferents show such adaptation. They are presumably all subject to the same cupula-response dynamics, unless one assumes a complex multimode cupula-de-

formation response. (As discussed in the chapter by Goldberg and Fernández in this *Handbook*, it is primarily among the irregularly discharging neurons that this adaptation is seen.) On the other hand, units located in the vestibular nuclei nearly all show a longer dominant response time τ_L and an overshoot in the postrotatory phase, similar to that seen in nystagmus response (163). Similar kinds of behavior but with shorter time constants are evident in units of the thalamus and may be related to evidence of adaptation in the pathways for subjective sensation of rotation (24).

Adaptation effects on the frequency response of perception are, as might be expected, evident only at very low frequencies (below 0.01 Hz) and consequently are not evident for normal physiological movements. The frequency response for subjective sensation is shown in Figure 9. The gain and phase relationships between the sinusoidal angular velocity of the head (input) and other perceived angular velocity or velocity of the slow phase or vestibular nystagmus (outputs) illustrates the role of the vestibular system in angular-velocity measurement. Over the midfrequency range (0.1–1.0 Hz) corresponding to most normal head move-

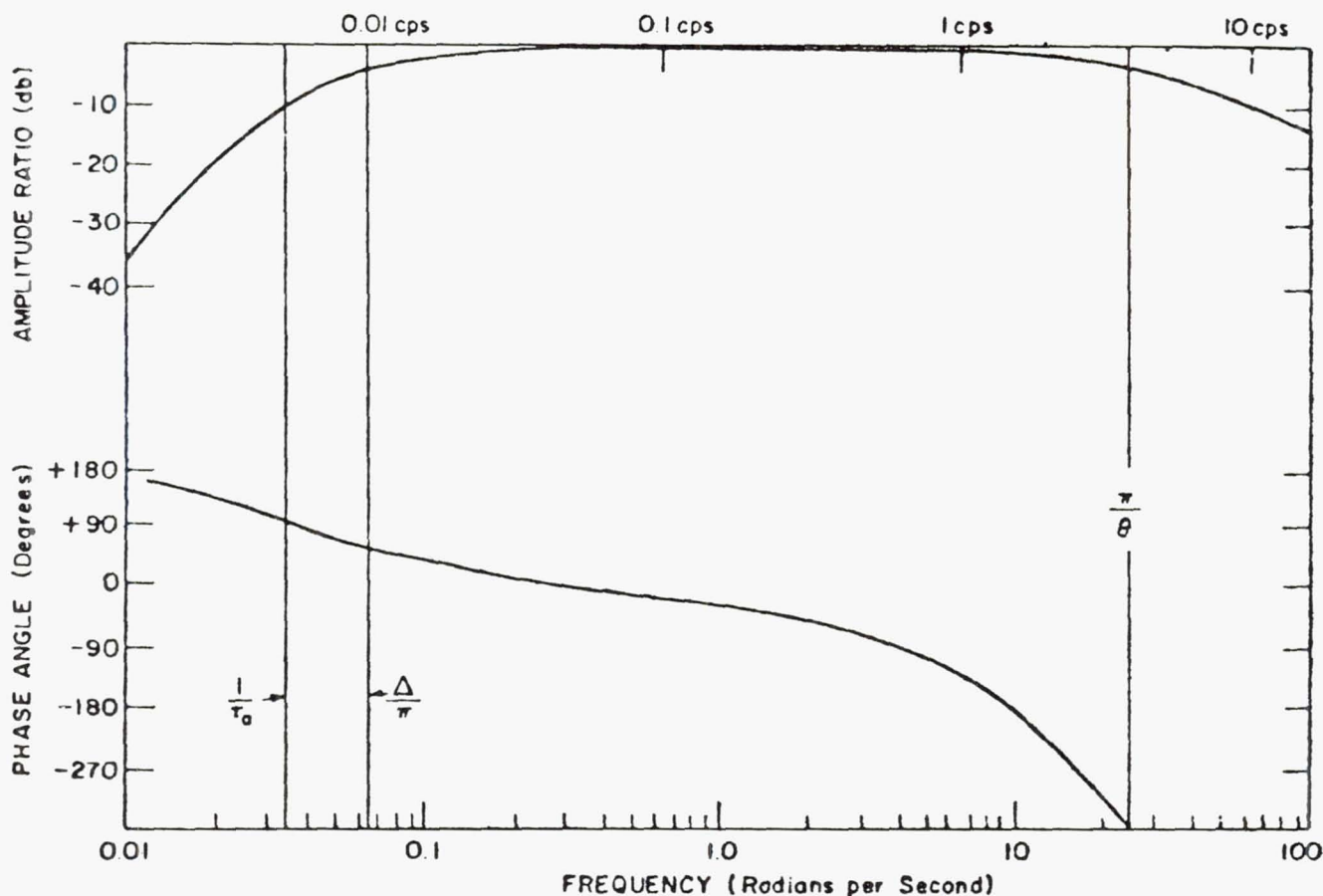


FIG. 9. Frequency response of adaptation model for subjective sensation during yaw angular motion: subjective angular velocity/input angular velocity equals $24.8e^{-0.3s}/(s + 25)(s + 0.0625)(s + .033)$. [From Young and Oman (178).]

ments, the gain is constant and the subjective and nystagmus velocities are roughly in phase with the stimulus. (The eye velocity, of course, is compensatory and opposite in direction to the head movement.) At higher frequencies above 1 Hz, there is evidence that vestibuloocular gain increases to fill in for the failure of pursuit tracking to maintain fixation at higher frequencies. Subjective sensation is virtually meaningless regarding phase at such high frequencies.

For frequencies below 0.1 Hz the gain declines, and the phase of the subjective and nystagmus velocity response exhibits a substantial lead relative to head velocity. Toward 0.01 Hz the response velocities are closer to being in phase with the input acceleration than with velocity, which is another way of representing the low-frequency inadequacy of the vestibular system in measuring angular velocity. The adaptation term leads to a phase lead of greater than 90° at extremely low frequencies (22).

Caloric and Alcohol Effects

The cupulae of the semicircular canals are normally maintained at a density very close to that of the surrounding endolymph, so that they serve as sensors for angular acceleration but are insensitive to linear acceleration. If absolute neutral buoyancy were maintained (cupula and endolymph of equal density), each semicircular canal afferent activity would be independent of orientation of that canal with respect to the gravity vector. In fact, individual canal units often show a gravity sensitivity in addition to their primary response to angular acceleration (103, 105). Presumably any small gravity-sensitive effects of the semicircular canals are either accounted for by central compensation based on otolith inputs or are of such minor influence that they are inconsequential. There are, however, two relatively common situations in which the delicate balance of density between cupula and endolymph is disrupted. One is the clinical test known as caloric stimulation and the other results from ingestion of alcohol.

Caloric stimulation, as introduced by Bárány (2) shortly after the turn of the century, remains among the principal tools of the otolaryngologist for diagnosis of peripheral labyrinthine disorders. The principal mechanism is essentially as described by Bárány. Irrigation of the outer ear by water or air warmer or colder than body temperature introduces a thermal gradient that in time reaches the endolymph of the lateral semicircular canal on the irrigated side. Warm fluid decreases the endolymphatic density (146), causing the now denser cupula to sag in the direction of gravity, as indicated schematically in Figure 10. If the head is placed so that the lateral semicircular canals lie other than in the horizontal plane, a component of gravity acts on the cupula to cause a pressure difference across it or a minor cupula displacement entirely analogous to the sort of cupula displacement resulting

from the physiological stimulus of angular acceleration. Detailed measurements and calculations (128, 145) have shown that the torque thresholds associated with generation of nystagmus are equivalent for caloric stimulation and for angular acceleration. This simple theory predicts that the magnitude of the nystagmic response (measured by slow-phase velocity of eye movements) should be proportional to the cosine of the angle between the semicircular canal and the gravity vector. Adaptation occurs to caloric stimuli in a manner somewhat like that to prolonged acceleration stimuli (13, 124). Furthermore some basis is pres-

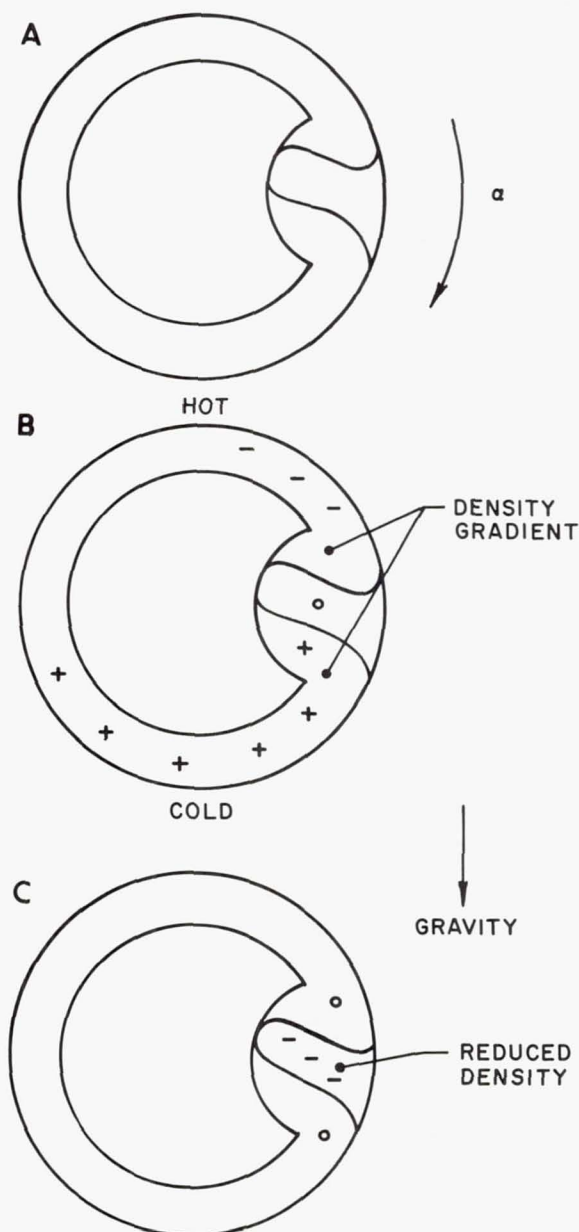


FIG. 10. Exaggerated cupula displacement during A: angular acceleration; B: caloric stimulation; C: first phase of alcohol nystagmus (PAN I).

ent for assuming that there is a direct thermal effect on nystagmus generation in addition to the influence of gravity. This assumption is supported by Coats and Smith (32), who indicate that the neutral points at which no nystagmus occurs are not found at head positions located at equal angles to those for peak responses (4). The case for direct thermal responses is weakened, however, by the measurements of Kellogg and Graybiel (97) and those of Oosterveld and Van der Laarse (130), who found that caloric responses disappeared during the zero-gravity phase of parabolic flight.

It must be recognized that the subjective sensation of spinning, frequently associated with caloric stimulation in the dark, is a bizarre and nonphysiological one. To maximize the caloric response, the head must be placed so that the lateral semicircular canals lie in a vertical plane. Consequently stimulation of these canals results in a semicircular canal signal, at least from one labyrinth, which indicates rotation about a horizontal axis. For unilateral stimulation this signal is not confirmed by the other labyrinth. Even for bilateral stimulation (hot water in one ear and cold in the other) the semicircular canal signals of rotation about a horizontal axis are in direct conflict with the signals from the utricular otolith organs, which indicate no change in head position with respect to the vertical. It is hardly surprising therefore that the magnitude and time constants of caloric nystagmus depend strongly on static head orientation beyond that necessary to account for the direct physical effect. Head orientation influences the effectiveness of otolith signals in suppressing both visual and semicircular canal inputs.

Alcohol is a commonly used drug that has at least two important effects on vestibular function, as reflected in nystagmic eye movements and perception of orientation. The anecdotal stories of a drunk being unable to walk a straight line (or, more correctly, a given curved line) are well known and reflect the underlying influence of alcohol on both the semicircular canals and on central compensation. The principal effect that has been studied is positional alcohol nystagmus (PAN). It has been known since the early work of Flourens (56) that, when the head is placed with one ear down, nystagmus beating in the direction of that ear takes place during periods of intoxication. The explanation for this phenomenon has only recently been made clear by Money and Myles (121), who demonstrated conclusively that the nystagmus generation (and presumably also the sensation of spinning) was associated with the direct physiological effect of alcohol in initially reducing the density of the cupula with respect to the surrounding endolymph. Once a density difference has been established, the influence of head position and gravity is the same as for caloric stimulation (see Figure 10C). A later effect, known as positional alcohol nystagmus II (PAN II), takes place 4-6 h after alcohol ingestion, when the

direction of the density difference between cupula and endolymph has been reversed. A further effect of alcohol on the central vestibular mechanism is perhaps to eliminate or to reduce the efficacy of central compensation for any minor imperfections or disorders in the peripheral labyrinthine system (8).

LINEAR MOTION AND GRAVITY

Nature of Linear Accelerometers

All linear accelerometers rely on the development of inertial reaction forces on a mass and measurement of either these forces or the displacements they create. Because it is essential to the appreciation of the perception of orientation during acceleration that the nature of an accelerometer and of inertial forces is thoroughly understood, an elementary review is included here.

If a 5-gram mass is held stationary in the hand, it requires an upward force on the mass of 5×980 dyn. This same force presses downward on the hand and can be used to weigh the object. If the mass is being accelerated upward by 980 cm/s^2 (1 *g*), then the force downward on the hand is $2 \times 5 \times 980$ dyn, or 10 gram-force (gf). If the mass is allowed to accelerate downward at 980 cm/s^2 , then no force is exerted on the supporting hand. If the mass is accelerated to the left at 1 *g* while still being supported to prevent it from falling, the force on the hand is the vector sum of 5 gf downward and 5 gf to the right, or $5 \times \sqrt{2}$ gf directed 45° to the right of down. The force on the support is, in each case, the product of the mass and the vector sum of gravity minus acceleration. This is referred to as the inertial reaction force. When this inertial force is divided by the mass, leaving only the vector ($\mathbf{g} - \mathbf{a}$), it is referred to as specific force. Unless the test mass, sometimes called the seismic mass, is surrounded by a fluid of equal density to it so that it remains neutrally buoyant, the action of a specific force would be to press it against its supporting members. These supporting members may be an instrument, springs, muscles, hair cells, or supporting cells.

The otolith organs are by no means the only linear accelerometers in the body. The head, with its point of suspension at the neck several centimeters below the center of mass, serves as an accelerometer. During forward accelerations it snaps back, sometimes with disastrous results. Our whole body serves as a seismic mass supported by the forces through our feet or contact forces from a seat. Thus when a pilot is forced down into his seat by centripetal acceleration during a tight turn, he can use the associated tactile forces to estimate his acceleration as he "flies by the seat of his pants." Our lungs, being considerably less dense than the surrounding tissue, also serve as a potential linear accelerometer. Because the buoyant forces are greater than the inertial reaction forces for the lungs, however, the displacement of the seismic mass is in the direction

of acceleration. If the cupulae of the semicircular canals are not precisely balanced so as to be of the same density as the surrounding endolymph, as appears to be true when they are infused with alcohol or heavy water, they can serve as the mass for a linear accelerometer and respond with a signal dependent on the orientation of the head with respect to gravity. As late as the nineteenth century it was assumed that human spatial orientation with respect to gravity was determined by shifts of brain tissue or blood in the head on tilting.

Among all the possible linear accelerometers in the human body that might be useful for orientation, the otolith organs play a unique role. As discussed in the chapter by Goldberg and Fernández in this *Handbook*, the utricular macula, with its collection of hair cells oriented in various polarizations, is primarily sensitive to components of specific force that are parallel to the local plane of the utricular membrane and produce shearing forces on the hair cells. Thus when the human head is pitched 25° forward from the fully erect position (carried in a normal orientation for walking) with the major planes of the utricle horizontal, the hair cells are in an ideal position to detect any linear acceleration in the horizontal plane. Because the otolithic membrane is displaced by the influence of specific force and not acceleration alone, it is also highly sensitive to the generation of any component of gravity lying along the polarization vectors of the hair cells. In particular, it is ideally suited to detect any tilt of the head forward or backward (pitch) or left or right (roll). Because the hair cells have no way of indicating whether they were displaced by the action of gravity or linear acceleration on the overlying membrane (and, indeed, the equivalence principle of Einstein states that no physical instrument can distinguish between these two equivalent accelerations), it seems clear that this one, approximately planar, accelerometer is faced with providing an ambiguous signal—one that cannot distinguish between a body being accelerated forward or one being pitched backward. Furthermore, the specification of the direction of specific force in a plane does not give a unique orientation of that plane relative to the specific force vector. As a trivial example, signals from the utricular otolith cannot be used to distinguish between right side up and upside down with the head in its normal position relative to the trunk.

The orientation information available from the sacculus is not to be overlooked, however. It is ideally suited for detecting vertical movements when the head is erect, or small rolling movements when the head is tilted at 90° to the vertical, or small pitch movements when the head is in the prone or supine position.

It might be argued that knowledge of the magnitude as well as direction of the specific force vector relative to the head would be sufficient to determine orientation, but even this is not valid. First of all, there are an infinite number of acceleration and gravitation

vector combinations that have a resultant vector magnitude of $1 g$. One simple combination is a forward acceleration at 9.8 m/s^2 combined with a free fall vertically at 9.8 m/s^2 . More practically, consider the specific force associated with a static head tilt of 1.5° to the right, which corresponds roughly to the threshold of detectable head tilt. The lateral component of acceleration is $g \sin 1.5 = 0.026 g$. The compressive component in the major plane of the utricles, lying in the plane of the sacculus, is $g \cos 1.5 = 0.9997 g$. This is indistinguishably different from $1 g$ and is not detectable by the sacculus units. Thus, based on information from the otolith organs alone, there would be no way of distinguishing between the tilt of 1.5° to the right or an acceleration of $0.026 g$ to the left. This ambiguity is a real one and can lead to a number of illusions of erroneous spatial orientation. It is discussed in *Ambiguity of Subjective Response to Acceleration*, p. 1046. The ambiguity can be resolved only by using other information, such as semicircular canal signals, that indicates whether or not the subject has been rotated at a suprathreshold rate. The ambiguity can also be cleared up by reference to the expectation of the possible motions that might have been imposed, to information from nonvestibular cues, or by expectation based on voluntary movement.

Static Orientation to Vertical

Two principal methods have been employed in determining the ability of a subject to judge his orientation relative to the vertical in the absence of visual orienting cues. In the nulling method the subject, seated in a tilting chair, is normally displaced from the vertical and is permitted to return himself to what he feels is the erect position. The accuracy with which the subject can return to the vertical depends on the length of time he was left in the tilted position: long durations lead to adaptation or undershoot on the return. The speed at which he was originally tilted (higher speeds lead to higher accuracies) and the smoothness of the allowable return also influence the results. When applied to threshold measurements, subjects were typically able to judge correctly when they were tilted away from their assumed vertical 75% of the time if the tilt exceeded approximately 2.2° (110). (To compare to linear acceleration thresholds, this corresponds to a lateral component of gravitation equal to $0.038 g$.)

Another widely used technique for judging the vertical from a tilted position is to have the subject align an illuminated line or rod to the judged vertical against a dark or homogeneous background. This use of the visual vertical allows the perceived angle of tilt to be tested without the complication of the dynamics of return associated with the nulling method. When the observer is seated in the upright position, this judgment can normally be made to within approximately $\pm 3^\circ$. Most importantly, this technique allows quanti-

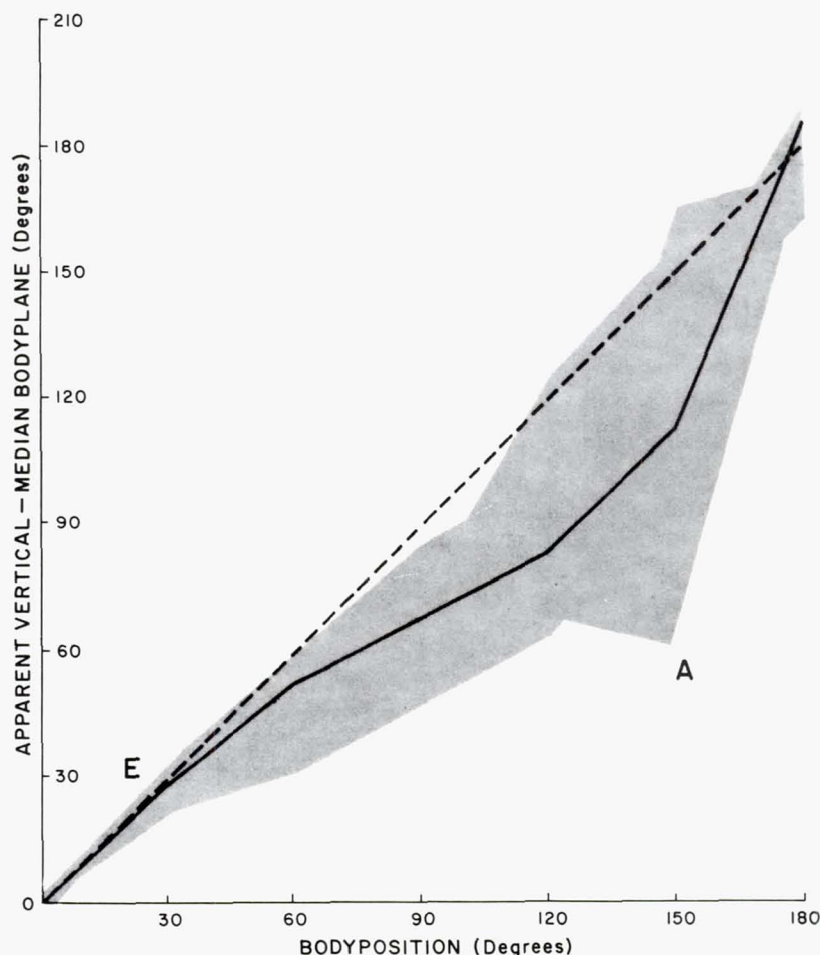
tative measurements of perceived tilt as a function of actual body tilt to be made at all body-tilt angles.

The estimation of body tilt with respect to the vertical, made by aligning a visible line to the perceived body axis without any other visual reference, might be thought to give the same indication of perceived tilt as the alignment of the line to the perceived vertical. Interestingly enough, the estimates of the body axis, although agreeing generally with the perceived vertical estimates for tilt angles up to 30° – 40° , continue to show an overestimation of body tilt for larger tilt angles. At 90° of pitch or roll, for example, when underestimation of body tilt is maximum, the perception of the tilt angle of the body axis is always overestimated. Nearly all of the quantitative work on orientation perception, however, uses the indication of the visual vertical, and it is on this basis that this discussion is based.

For small tilt angles of the whole body about the x -axis, a truly vertical line appears tilted in the direction of body tilt, and in estimating the vertical with this line, many subjects tend to set it to a tilt opposite the direction of the body tilt. This overestimation is referred to as the Müller or E effect (123). As tilt angles increase beyond 30° , however, many subjects

indicate a reversal in the direction of error and underestimate the amount of tilt. The line is then set tilted in the same direction as the actual body tilt. This is known as the Aubert or A effect (1). Not only does the principal error change as a function of the body angle, but the variability in making these estimates increases significantly for tilt angles above 30° . The range of individual differences for the A and E effects are shown in Figure 11 for 13 subjects whose apparent tilt angles are plotted. The increase in variability of the judgment of the vertical made from positions other than the upright has been associated with a dependence on saccular rather than utricular cues. As the tilt magnitude ϕ approaches 90° , the theoretical sensitivity of the utricular signals $g \cos \phi$ to changes in body angle approaches zero. The magnitude of the deviations from the true tilt angle may be varied on a centrifuge by increasing the strength of the specific force acting on the otoliths. Although ocular counter-torsion occurs during the head tilt, it is certainly not the cause of the A effect. First of all, the ocular counter-torsion would tend to reduce the angle of the true vertical relative to the vertical axis of the retina and consequently introduce errors in the opposite direction to the A effect, if no compensation was made

FIG. 11. Measurement of relative inclination of luminous line that subjects set to apparent vertical when they are tilted laterally. Range and median shown for 13 subjects. Overestimation of tilt at small angles is the E or Müller effect shown by some subjects. The underestimation A or Aubert effect at large angles is more pronounced. [Adapted from Bischoff (9), using data of Udo de Haes (157).]



for eye movements. Fischer (51, 52) concluded, on the basis of direct measurements of ocular counterrolling and the A effect, that the phenomena were independent. Clearly, large angles of tilt underestimation up to 45° could not be explained on the basis of ocular countertorsion, which normally does not exceed 6° – 8° . Most conclusively, Fischer (53) demonstrated the existence of the A effect but no ocular counterrolling in a patient with no labyrinthine function.

The E effect for tilts up to 30° is not limited to visual orientation of a line. Blindfolded subjects showed similar overestimation using a palpated rod to indicate the vertical (10). A similar technique was used in Skylab for judging orientation relative to the spacecraft (70). Auditory localization is also affected by the tilt in the same manner (153). Reviews of the various studies of the A and E effect in a 1-g field are given by Howard and Templeton (90), Guedry (76), and Schöne (141).

Similar consistent patterns of overestimation of small tilt angles and underestimation of large tilt angles also appear for pitch about the y-axis (141). There have been several attempts to explain the mechanisms of vestibular activity underlying the A and E effects. In particular, Schöne and his colleagues have supported the notion that the shear component of specific force lying in the plane of the utricular macula is primarily responsible for the illusion of tilt. At any given head pitch orientation θ and roll angle ϕ with respect to the vertical, the lateral and forward shear components in the plane of the utricle are given by the components

$$f_{y0} = \cos(\theta - \theta_u) \sin \phi$$

and

$$f_{x0} = \sin(\theta - \theta_u) \cos \phi$$

θ_u represents the pitch inclination of the major plane of the utricle with respect to the anatomical horizontal and is usually assumed in the human being to be pitched up or back by 25° – 30° relative to the anatomic horizontal plane. Thus no forward shear component is present when the head is pitched forward θ_u , and all roll-tilt components, f_y , are maximized when the head is in the θ_u pitch-forward position at the time of the roll. By setting the perceived tilt angle proportional to f_y for roll and proportional to f_x with a bias component of θ_u for pitch, a rough approximation to the results of tilting in a static 1-g field is achieved. To fully understand the influence of compressive forces on the utricular macula and of the role played by the sacculus, however, it is necessary to examine the illusions of pitch taken under higher gravity loads. This is accomplished by placing subjects in gondolas at the end of a centrifuge arm and rotating at constant angular velocities so as to achieve a static gravito-inertial specific force vector greater than 1 g. Perceived angle of tilt was measured when subjects were placed at various pitch and roll angles with respect to this resultant g

vector. Different combinations of body tilt and resultant g -vector magnitude could be used to independently vary the shear components in the plane of the utricle and the compressive components on the utricle. (The latter lie approximately in the planes of principal sensitivity of hair cells for the sacculus.) It became clear that the magnitude of the shear component in the lateral plane in the centrifuge experiments was not sufficient to predict the perceived tilt angle. Indicated lateral tilt at various specific force levels between 1 g and 2 g is shown in Figure 12. In all cases an increase in compressive force, keeping the utricular shear component fixed, led to an increased angle of tilt. A mere increase in utricular compressive component alone when no utricular shear component is present did not result in any change in perception of body pitch or roll (36, 140).

The perceived pitch or roll angle depends on the specific force perpendicular to the utricular plane as well as that in the plane. The utricular shear theory, although very useful for explaining perceived tilt, ocular countertorsion, and acceleration thresholds for small deviations from the upright position, generally is not valid. Several hypotheses have been considered to expand it to include the results of experiments performed at increased gravitation levels. One line of thinking maintains the primacy of utricular signals in determining orientation with respect to the vertical, but it includes nonlinearities. Among these nonlinearities is the possibility that the utricular shear signal saturates as the stimulus approaches 1 g in the plane

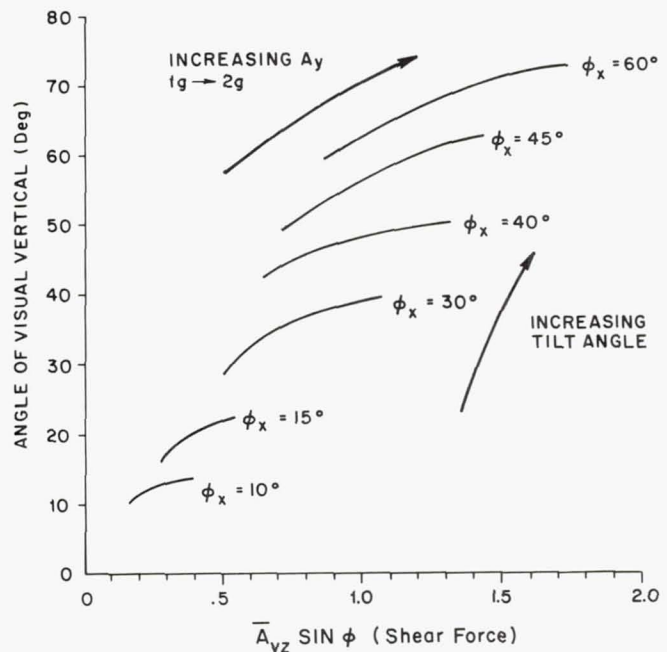


FIG. 12. Measurements of angle of a line set to apparent vertical, plotted against lateral component of specific force for various body tilt angles (ϕ) and g levels. Apparent tilt varies according to shear force; it also increases with compressive force component. [Adapted from Correia et al. (36, 37).]

of the utricles. Clearly, head tilts in the normal 1- g environment never produce sustained stimuli exceeding 1 g , and one might argue that the transducer was not built to handle loads above 1 g . Another explanation, based on physical mechanisms of displacement of the otolith membrane over the utricular macula, is simple and appealing. Correia et al. (37) pointed out that, once the hair cells were bent by a shear component of specific force, any subsequent compressive component would result in further bending of the hair cells, thus increasing the afferent signal and presumably increasing the perceived tilt. Benson and Barnes (5) developed this theory mathematically and showed that indeed single utricular afferent signals, as measured during head tilt and on centrifuges, could be explained on the basis of this combined loading. This notion is also consistent with measurements of ocular countertorsion that show a similar pattern of relatively large gain for tilt angles up to 60° and much reduced gain for tilts beyond that in a 1- g field. Others have emphasized the role of somatosensory cues in accounting for the deviation of the visual vertical from the predictions of the shear hypothesis (102). All of these theories neglect the findings, now reasonably conclusively demonstrated, however, that stimulation of the sacculus plays a role in orientation in animals. Direct saccular stimulation results in eye deviations (57), and it has also been shown that the saccular signals respond to steady-state orientation about the vertical (50).

One general algorithm for prediction of overestimation and underestimation of pitch and roll at acceleration levels equal to and exceeding 1 g has been worked out by Ormsby and Young (132). The result of this theory is a simple diagram for predicting A and E effects in any gravity field, as shown in Figure 13. The heavy line pitched up by an angle of θ from the vertical when the head is in a normal erect position represents the dominant plane of the utricles. As usual, θ is taken to be 25° – 30° . The net gravito-inertial or specific force vector is indicated by \mathbf{f} . It may have components perpendicular to the plane of the utricle, f_z , in the lateral plane, f_y , or longitudinally in the plane of the utricle, f_x . The tilt is taken to be an underestimation (category A, Aubert illusion) when the compressive component, f_z , is less than $1\text{ }g \times \cos \theta$, which corresponds to the compressive component present when the head is erect in a 1- g field. Similarly if the compressive force is greater than $g \cos \theta$, a Müller illusion or overestimation of tilt occurs. In category N, when the compressive component of f_z is exactly equal to $g \cos \theta$, the veridical pitch or roll is presumed to be felt. The actual angle of perceived tilt that is predicted for any head orientation is calculated by the nonlinear transformation of f_z to \tilde{f}_z , shown in Figure 14. The components of f_x and f_y are carried forward without nonlinearity and combined as a vector sum with \tilde{f}_z to produce the estimated vector representing the vertical. Figure 14 implies that information from the sacculus

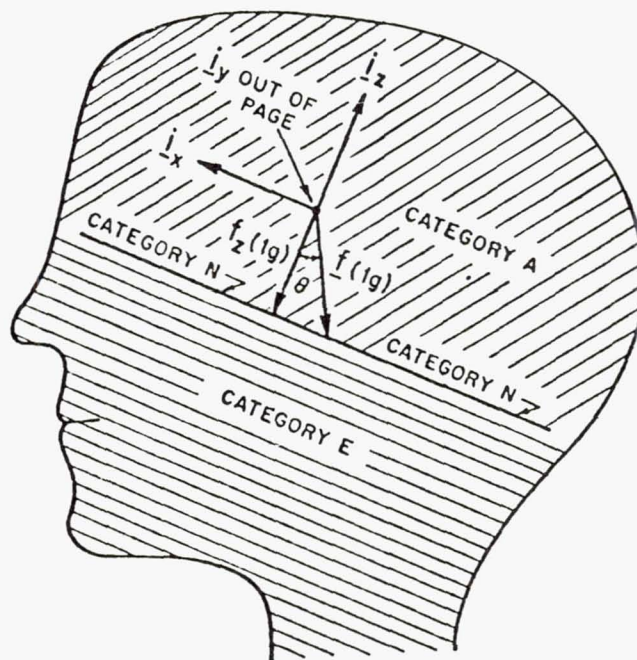


FIG. 13. Schematic representation of perceived pitch categories in various gravitational fields. The z -axis component of specific force determines whether actual pitch is underestimated (category A) or overestimated (category E). [From Ormsby and Young (132).]

is nonlinearly transformed, with a saturation at levels of saccular stimulation exceeding those that correspond to vertical accelerations greater than 1 g , and with a slope of less than unity (range 0.6–1) for acceleration components less than 1 g . Although the results of a number of experiments are not predicted adequately by this theory, especially for head tilts greater than 90° from the specific force vector, it does encompass many of the test results exceeding 1 g , as indicated in Figure 15. According to the theory, the utricular shear for lateral tilt is dominant in determining orientation for small tilts away from the vertical, wherein the stimulation to the sacculus remains virtually constant. Saccular information, however, cannot be ignored for larger tilt angles or for higher acceleration levels. On the other hand, information from the sacculus is not transformed without error. It results in an ambiguity for vertical accelerations that produce net downward components of \mathbf{f} greater than that which would be expected in a 1- g field. We refer again to this ambiguity in the interpretation of saccular information in treating the case of perception of orientation during vertical oscillations.

An alternative scheme for combining utricular and saccular information has been proposed by Schöne (141), and this scheme appears to match experimental data in a 1- g field at all angles. In that formulation, saccular signals merely switch the utilization of the utricular shear signal when the head is tilted more than 90° .

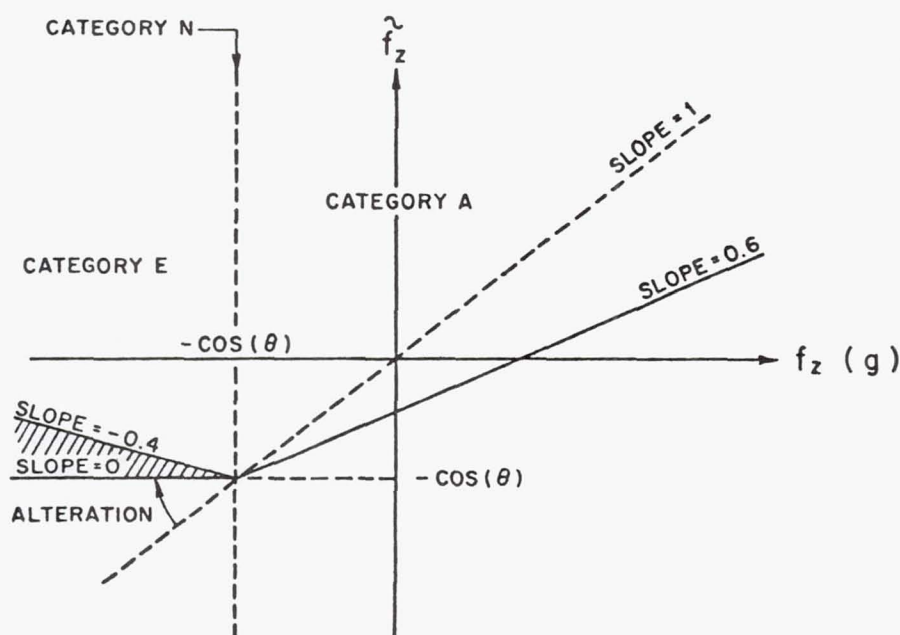


FIG. 14. Alteration of z -component of specific force to yield observed errors in perceived pitch. See text for explanation. [From Ormsby and Young (132).]

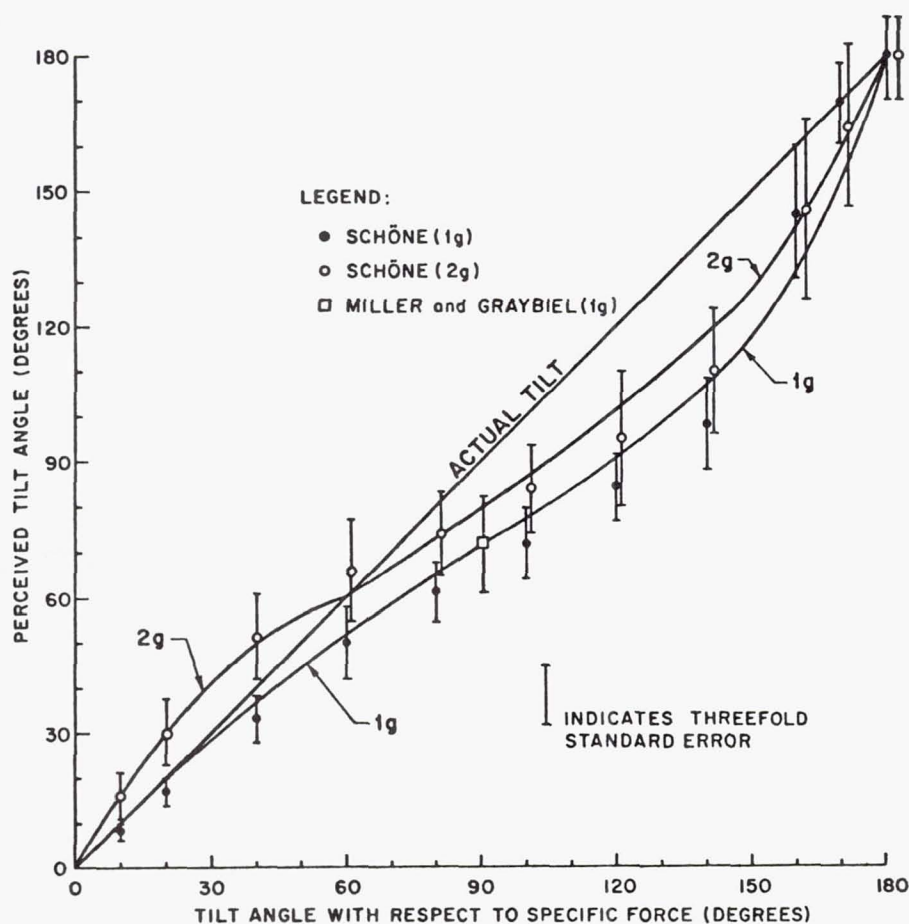


FIG. 15. Model predictions for perceived lateral tilt angle as function of actual tilt (as in Fig. 11) compared with data taken at 1 g and 2 g . [From Ormsby and Young (132).]

Contributions of Nonlabyrinthine Sensors to Perception of Tilt

In all of the tilting experiments described above, subjects received consistent cues from the various sensory systems. Confirming cues emanated from the otolith organs, from surface and deep pressure tactile receptors, and occasionally from proprioceptive or kinesthetic receptors activated by the muscular effort required to maintain limb, head, or trunk postural stability in the tilt position. The preceding discussion emphasized the role of the shear component of specific force, especially on the utricular otolith, but also on the sacculus. Several techniques have been used to isolate the relative contributions of the labyrinthine and nonlabyrinthine (nonvisual) cues (76). Tactile and proprioceptive cues can be reduced, if not entirely eliminated, by various methods discussed in *Reduced Somatosensory Cues*, this page. Labyrinthine cues can be eliminated through the use of labyrinthine defective subjects in experiments. Static otolith cues are temporarily eliminated during free fall, in aircraft parabolic flight, or in spacecraft. Finally, somatosensory and proprioceptive cues can be augmented artificially by various techniques.

Reduced Somatosensory Cues

The tactile and proprioceptive cues afforded a normal subject may be reduced by using local anesthesia on the soles of the feet or the buttocks to eliminate support cues. Others have experimented with subjects who have suffered from spinal lesions, resulting in the interruption of afferent information from the parts of the body where support was provided. Most commonly, however, tactile cues were not eliminated but rather were spread out over a very large contact area, so that the peak pressure at any one part of the skin or over any one of the deep pressure sensors was kept relatively small. These latter methods either used form-fitting molds or were performed underwater. It should be clear that even in a neutral buoyancy underwater experiment, the average pressure on the lower side of the subject is greater than that on his upper side. The pressure differences are relatively small, however and may be imperceptible. When normal subjects are deprived of the usual somatosensory cues during tilt experiments, their average perception of tilt does not change noticeably, but the variance of their estimates of orientation increases markedly. Brown (20) carried out extensive experiments on the ability to perceive orientation with respect to the vertical when underwater and to right one's self with respect to the vertical. Errors in estimation compared with measurements taken with the normal-tilt chair support structure increased markedly, especially when the estimations were made with the head down or the face up. Especially large errors occurred when the tilt angle was more than 60° from the erect position in pitch or roll. Schöne (139) found similar results in his

underwater experiments wherein subjects indicated their orientation to the vertical by adjusting a line in the usual manner. With water immersion, subjects exhibited slightly greater E and A phenomena than when suspended from straps. The principal difference is the increased variance when somatosensory cues are reduced. Both Schöne and Brown found that the position with the head down led to the largest errors. When tests of alignment to the vertical were carried out on a centrifuge with normal subjects immersed in water to the neck, Graybiel et al. (71) found a relatively small reduction in perceived tilt relative to the same tasks carried out under dry conditions, as shown in Figure 16. Schock (138) also found that subjects attempting to set luminous rods to the vertical at various tilt angles underwater showed approximately twice the variability as they did when they were dry. Tactile cues do not normally introduce any major consistent bias in judgments that are primarily based on labyrinthine sensory information, particularly within $\pm 60^\circ$ of the erect orientation. They do, however, reduce the variability of such judgments.

Labyrinthine Defective Subjects

Unlike visual or auditory senses where the distal stimuli are easily removed by closing the eyes or blocking the ears, the vestibular sense organs of normal subjects cannot normally be shut off. Even in the absence of any linear or angular acceleration cues, the unmodulated resting discharge signals to the central nervous system that no measurable acceleration has

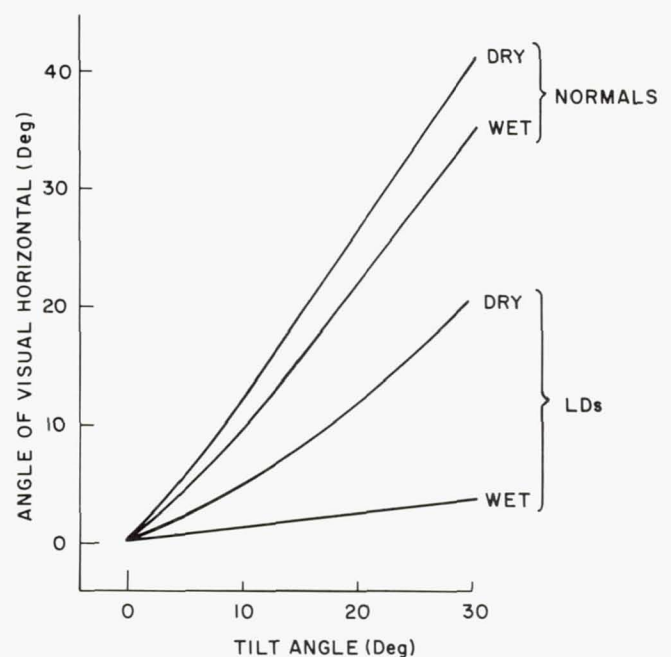


FIG. 16. Comparison between settings of line to perceived horizontal by normals and labyrinthine defectives (LDs) in air and when tactile cues were reduced by submersion in water. [Adapted from Graybiel (71).]

taken place. The importance of this null signal is amply demonstrated by the generation of optokinetic afternystagmus (33) and by the production of motion sickness symptoms from visual stimuli alone in stationary subjects with functioning vestibular systems (18, 119). Consequently, to investigate the role of nonvestibular cues in determining orientation, and in particular for perceiving tilt, wide use has been made of subjects with bilateral loss of vestibular function. Loss of bilateral labyrinthine function is not uncommon among deaf subjects and may be the result of a number of disease entities or surgical intervention to treat severe auditory or vestibular problems. The absence of semicircular canal function is demonstrated by the absence of nystagmus during extreme caloric stimuli, and the absence of otolith function is demonstrated by a minimal ocular counterrolling response to head tilt.

Labyrinthine-defective (LD) subjects show a threshold to linear acceleration of the range of 15–26 cm/s^2 compared to thresholds in normals of 2–5 cm/s^2 . By way of contrast, removing substantial amounts of somatosensory input from normals by testing during water immersion raised thresholds to 4–7 cm/s^2 , and thresholds of subjects with high spinal lesions interfering with somatosensory afferent feedback were also in the range of 4–8 cm/s^2 (164). The lower curves in Figure 16 show that the perceived tilt for LDs during rotation on a centrifuge is about one-half that for the normal subject population. Furthermore the LD subjects, when deprived of their principal somatosensory information by water immersion, show a marked drop in the perceived tilt angle (71).

It is somewhat puzzling to note that the LD subjects consistently underestimate the angle of tilt with respect to the resultant vector on a centrifuge, whereas the normal subjects perform reasonably well, except for the A and E effects. On a tilt chair, however, where the direction of the specific force vector with respect to the head varies as much as it does for the centrifuge experiments, LD subjects are able to return themselves to the postural upright position with about the same accuracy as do normal subjects (30). Apparently the LD subjects, although much less accurate than normals in estimating the actual angle of tilt with respect to a force vector, are roughly equivalent in perception of tilt at one critically important angle—that which corresponds to maintenance of the head in the erect position. Not unexpectedly, when LD subjects are given a sufficient number of trials to practice returning themselves to the vertical from the tilt positions maintained for various periods of time, they do nearly as well as normals. Mean deviations for normals are of the order of 1.5° compared to 2.5° for LDs (143).

Experiments on perception of the vertical with tilted LD subjects afforded an opportunity to test the hypothesis that the A and E effects were associated with nonlinearities in the otolith organs. The A and E effects exist for LD subjects as well as normal subjects,

making it clear that the otolith organs are not solely responsible for these systematic errors in perception of tilt. The magnitude of the effects, however, are far greater for the LD subjects than for the normals, and the transition from E to A phenomena occurs at smaller tilt angles with LDs than with normals (118).

Experiments on LD subjects, as well as those concerned with reduction of somatosensory cues for normal subjects, indicate that in the normal subject there exists a high degree of redundancy between somatosensory cues and otolith cues concerning orientation to the vertical. This redundancy leads, in the normal human being, to high repeatability in orientation and low thresholds for detection of acceleration or tilt. The illusions of underestimation of tilt are not uniquely attributable to the otolith organs or to the somatosensory system. In the absence of somatosensory cues, the variability in judgment increases. In the absence of labyrinthine cues, somatosensory cues are reasonably effective in the region of the upright head-erect position, but they fail for head angles more than 10° – 20° from the vertical.

Amplified Somatosensory or Postural Cues

Somatosensory signals may be distinguished from vestibular cues by independently altering the strength of the former. Relatively enhanced sensory cues can be provided during zero-gravity flight, when the otolith organs provide no indication of static orientation. A tactile indication of a local “down” reference can be established by pressing a subject down to his couch or with his feet against a surface using elastic tethers. Graybiel et al. (69) experimented with astronauts in the Gemini flights, and similar experiments were carried out by Graybiel et al. (70) on Skylab to determine the ability to maintain a sensation of body orientation with respect to the spacecraft in the absence of vestibular stimulation and visual cues. Settings of an apparent horizontal line with respect to the body axis were consistent and showed no obvious deviations, leading to the conclusion that the remaining tactile, proprioceptive, and kinesthetic cues, transmitted through the chair support, were sufficient to define this reference frame. Recent observations on the ability to voluntarily change one’s sense of the direction of a local vertical during free fall has indicated that for many subjects tactile cues can be used for this purpose in the absence of otolith cues.

The voluntary muscular effort required to avoid falling can provide sensory cues that also contribute to judgment of orientation. Most of the tilt experiments discussed above were carried out with a seated subject. When similar experiments were performed with the subject standing, using tilts of up to 20° to the right and left of the vertical, Clark and Graybiel (30) found that neither their LD subjects nor normal observers showed any significant errors in tilt perception, although the tilt angles were somewhat small.

The notion that the muscular activity required to avoid falling influences perception of orientation is strengthened by experiments in which this activity is caused to be altered without head tilt. Uneven application of weights can produce asymmetrical forces on the head or body (98, 99, 137), which is resisted by asymmetrical muscle tone. The muscle tension can be sensed by muscle spindles and by Golgi tendon organs. Wapner et al. (166, 167) showed that galvanic stimulation to the muscles to change muscular activity could also produce an asymmetrical tone. Each time the perception of body tilt was similarly influenced, just as though the muscular tone was necessary to avoid falling when tilted. Asymmetrical tactile cues have been used recently in aircraft flight simulators to provide the illusion of tilt or sustained acceleration (100). The application of asymmetrical tactile cues is achieved by differential elevation of various panels of a seat and back rest. The perception of increased magnitude rather than direction of the gravito-inertial acceleration is achieved by contouring the support so that all of the body's weight is taken on two high-pressure points, the ischial tuberosities.

The perception of orientation with respect to the vertical in the normal subject is thus seen to be modified somewhat by manipulation of the somatosensory input. In the absence of such cues, judgments are frequently variable. The tendency to make great errors in judgment as one approaches the otolithic blind spot in the head-down position is particularly apparent. It is of interest to note that no such blind spot appears obvious in examination of the neurophysiological data. Representation of the changes in acceleration from any initial head position appears to be adequately present both in the first-order afferents from utricular and saccular units (50, 155) and in the units recorded in the vestibular nucleus. Certain directions of linear acceleration sensitivity do, however, appear to be preferentially represented at the level of the vestibular nucleus in the cat (41).

DYNAMIC RESPONSE OF OTOLITH SYSTEM

Ambiguity of Subjective Response to Acceleration

Horizontal-axis linear accelerations may be looked on as the vector sum of two orthogonal components of specific force, downward directed gravity and horizontal force opposite to acceleration. The total gravito-inertial vector, however, which is the vector sum of these two forces, swings through an arc just as a pendulum would during horizontal acceleration of its pivot. It is well known that the semicircular canals as well as the otolith organs respond to such rotating linear acceleration vectors (4) and that nystagmic eye movement and subjective rotation are elicited by these rotating stimuli. It is therefore not a simple matter to distinguish between the influence of the rotating vector and the influence of the time-varying horizontal

component. Nor is it obvious whether the response that should be sought is one of perceived horizontal motion or perceived tilt with respect to the rotating vector. This difficulty can be overcome on earth by testing with dynamic linear accelerations along a vertical axis. In this case there is no rotation of the gravito-inertial vector, but all stimuli must take place about a 1-g bias level that may influence the results. The use of a centrifuge to build up horizontal components of acceleration presents additional problems. Either the subject is fixed in orientation with respect to the vertical, in which case the gravito-inertial vector rotates as in the case of the lateral oscillations, or he is in a pendulous swinging gondola that remains aligned with the gravito-inertial vector but which must tilt in order to do so, thereby stimulating the semicircular canals. Finally, transient linear accelerations normally take place beginning from a 1-g bias. Only during brief exposures in the zero-gravity portion of parabolic flight or during linear acceleration experiments carried out within an orbiting spacecraft can this initial bias be removed.

When performing open-loop or magnitude estimation tasks, it is by no means clear what response should be sought from the subject. For horizontal accelerations, various investigators have attempted to elicit responses of subjective displacement or subjective acceleration. Most success has been achieved by asking subjects to respond with subjective linear velocity. In its simplest form this comes down to a judgment of "which way am I moving," which is particularly useful for determining the phase relationships between imposed and subjective velocity. The occurrence of the alternate subjective orientation response of pitching or rolling in response to horizontal acceleration depends to a great extent on mental set and the perceived degrees of freedom of the stimulating device. Sometimes after prolonged oscillation in the horizontal plane the initial perception of pure translation changes to one involving a strong perception of tilt during the peak acceleration phases at the extremes of the oscillation (133). As is true in studies of the rotation-sensing system, many of these difficulties in reporting techniques can be overcome by using the nulling method, in which the subject attempts to maintain himself stationary in space through closed-loop control.

Linear Acceleration Steps

When a seated subject, deprived of visual cues, is accelerated in a horizontal direction along his x , y , or z head axis, the time to correctly detect the acceleration rises as the acceleration magnitude is reduced for accelerations below approximately 0.05 g (0.5 m/s^2). The data reported by Meiry (112) for accelerations along the x -axis are shown in Figure 17. The model predictions indicated by the solid line follow from a theoretical model of the form

$$a_{x_e} (1 + 0.07e^{-1.5t} - 1.07e^{-0.1t}) = \text{constant}$$

where a_{x_e} is the component of linear acceleration in the plane of the utricular macula and t is the time required to detect the constant acceleration. Meiry assumed that the direction was based exclusively on shear forces applied to the utricle, and he neglected the possible use of saccular information. Thresholds were extrapolated from these data and taken to be 0.01 g for the supine position (z -axis acceleration) and 0.006 g for the head-upright position. Meiry assumed that the absolute threshold, which would be reached for accelerations in the plane of the otolith with the head tilted forward 25° – 30° , would be approximately 0.005 g .

A comparable set of experiments, measuring time to detect linear acceleration in the vertical axis and taking into account the 1- g bias, was carried out by Melvill-Jones and Young (117). The times to detect the onset of acceleration as a function of acceleration magnitude, shown in Figure 18, were remarkably similar for the vertical accelerations, indicating that the rotation of the linear acceleration vector and consequent stimulation of the semicircular canals was probably not an important factor in the response to horizontal acceleration. A convenient method of viewing these data is to recognize the roughly hyperbolic shape of the latency time-versus-acceleration curves and, allowing for the existence of some minimum reaction time t_r , express the curves in the form

$$t = B/a + t_r$$

where t is the time to detect an acceleration step of magnitude a and B is the velocity constant, corresponding to the velocity that must be reached during brief linear accelerations before they are noticed. B was found to be 21.6 cm/s (± 2.6) and 22.6 cm/s (± 1.3) for vertical and horizontal acceleration, respectively, both with the head erect. [Horizontal acceleration with subject supine raised the constant to 32.4 cm/s (± 2.0).] The calculated threshold for vertical accel-

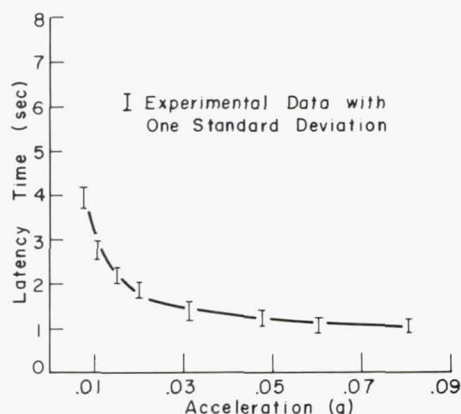


FIG. 17. Latency to detection of step of horizontal linear acceleration for subjects upright. See text for equation of model. [From Meiry (112).]

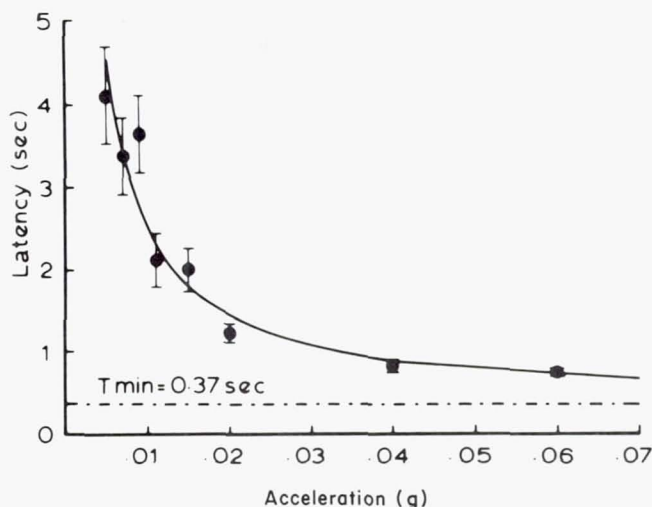


FIG. 18. Latency to detection of steps of vertical linear acceleration for subjects upright. Model is regression hyperbola for 8 subjects, yielding minimum response time of 0.37 s and velocity constant of 0.022 g -s. [From Melvill-Jones and Young (117).]

ation, stimulating primarily saccular receptors, is nearly the same as that extrapolated for horizontal accelerations stimulating the utricular receptors. What is significantly different between the two axes, however, is the presence of a high degree of ambiguity and confusion in judging the direction of acceleration in vertical motion. Despite the brief latency times to detection of motion for the vertical, subjects were frequently wrong in their judgment of the direction in which they were moving. Frequently subjects who are given step or sinusoidal accelerations in the vertical direction are totally incapable of judging the direction of their current motion, although they are able to detect accelerations (109, 117). Because saccular units are presumed to have roughly the same sensitivity as utricular units to linear acceleration along their axes of polarization (50, 169), this threshold or sensitivity difference cannot be ascribed to the end organ, but a central nervous system integrative process must be investigated for an explanation. This difference in the treatment of saccular and utricular information is implicit in the static orientation model discussed in *Static Orientation to Vertical*, p. 1039.

Sinusoidal Linear Acceleration

Mach (106) reported that the thresholds to detection of vertical periodic motion on a seesaw-type device ranged between 10 and 12 cm/s² (0.01–0.012 g) for himself and also for his technical assistant (87). A table of these thresholds, presented by Guedry (76), indicates a clustering of measures in the region of 0.005–0.01 g for oscillations in the 2-to-9-s period range. One of the remarkable aspects of perception of linear oscillation is the normally unexpected occurrence of a substantial amount of phase lead at lower frequencies. Although there is not a complete overlap

and agreement of data among investigators who have performed linear accelerations (94, 112, 165), there is agreement that the phase lag between stimulus velocity and perceived velocity increases with frequency. The phase relation for horizontal linear acceleration along the x -axis is shown in Figure 19. Reliable subjective judgment of magnitude is much more difficult to obtain. Walsh was able to demonstrate the erroneous phase relationship to his subjects dramatically by having them open their eyes after having made a judgment of their direction of travel and thereby determine whether their perception was leading or lagging the actual motion.

The low-frequency phase lead and high-frequency phase lag implied by the data of Figure 19 are consistent with a linear transfer function relating the perception of linear velocity to the actual horizontal linear velocity given by

$$\frac{\text{perceived velocity}}{\text{actual velocity}} = \frac{1.5(s + 0.076)}{(s + 0.19)(s + 1.5)}$$

The dominant time constant in this model (5.3 s) cannot be attributed to the mechanical events at the

otolith organs by analogy to the relationship of rotation sensation to cupula return dynamics. The only direct measurements of otolith displacements during oscillation, taken by deVries (42) in the fish, indicate an extremely fast reacting system with dominant time constants of the order of 0.005 s. Direct recording of first-order afferent units from the utricular and saccular maculae showed substantial sensitivity up to at least 2 Hz. Fernández and Goldberg (50) fitted the frequency response data with a transfer function having a dominant first-order lag-time constant in the range of 5 to 30 ms. (An additional long-adaptation time constant was highly variable among units.) Obviously the long integration times shown in the perception data must be found other than at the end organ. Some evidence suggests that much of the processing of otolith signals takes place by the level of the vestibular nuclei, where substantial phase lags have been reported. The limited data reported by Melvill-Jones and Milsum (115) on frequency response of cat vestibular nuclei to linear sinusoidal oscillation indicates a substantial buildup of phase lag over the frequency range of 0.1–2.0 Hz.

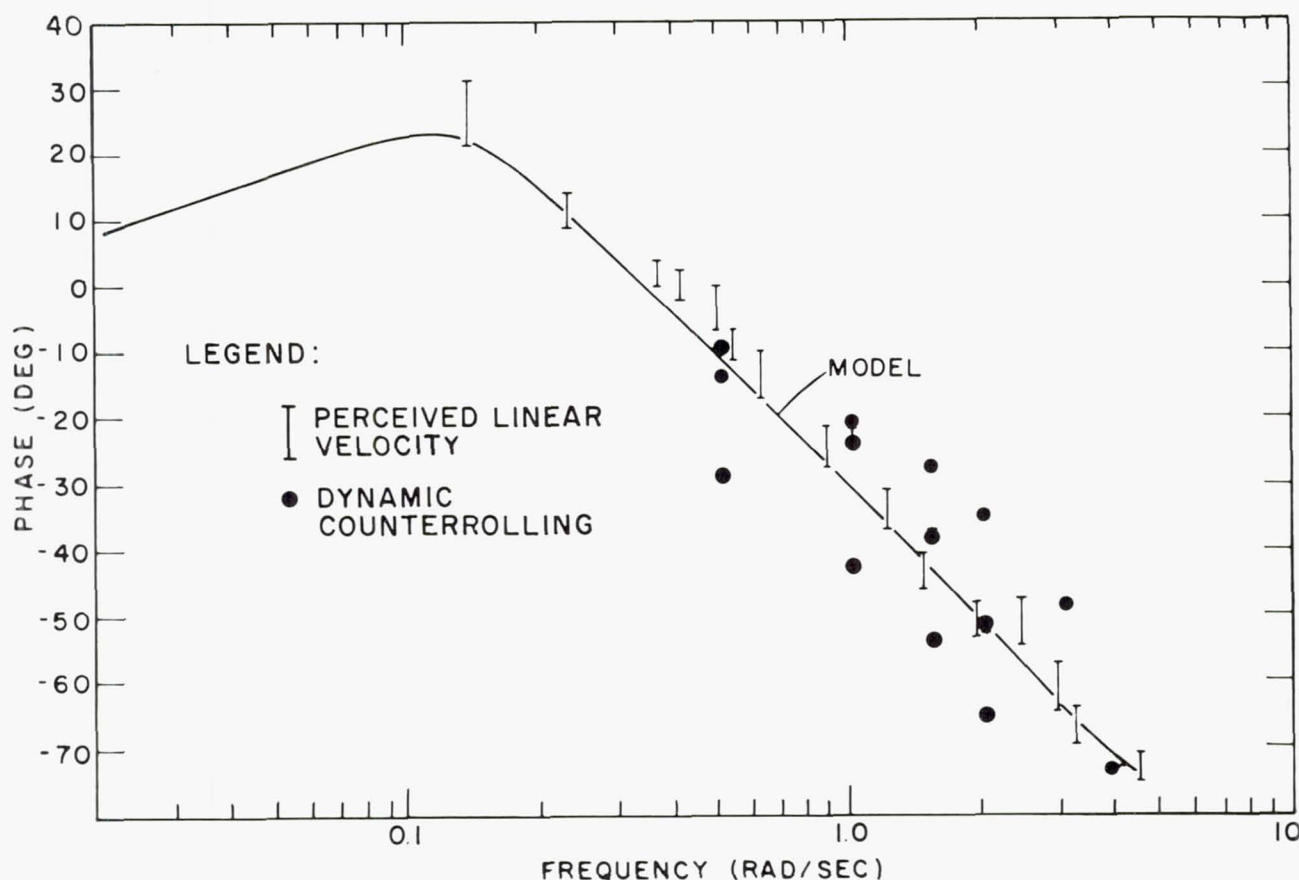


FIG. 19. Phase angle frequency response for perceived velocity vs. input velocity. Vertical lines showing ± 1 SD. [Dynamic ocular counterrolling vs. lateral specific force, measured by Kellogg (96a). From Young and Meiry (177a).]

COMBINED ROTATIONAL AND LINEAR ACCELERATIONS

Consistent and Inconsistent Vestibular Signals

Although most of the acceleration patterns discussed thus far involve stimulation of either the semicircular canals or the otoliths, the patterns of head movements encountered in daily life normally involve simultaneous stimulation of both types of labyrinthine organs. The simultaneous acceleration stimulation may be either consistent or conflicting. Of course all of the sensory cues are, in a certain sense, consistent if one takes into account the physical stimulation situation and the dynamic response of the sensors. The term *consistent*, however, may be restricted to the simple situations in which the interpretation of perceived motion may be reached by considering current semicircular canal output signals to represent head angular velocity and current otolith signals to represent direction of the specific force vector. A simple example of consistent cues is a rapid rolling motion of the head from the upright position 90° toward the right shoulder. If the movement is completed in less than 2 s or so, the vertical semicircular canals indicate approximately the correct instantaneous head-roll angle velocity, which can be integrated centrally to calculate the change in head-roll angle. This change in head angle of 90° is consistent with the short-term

measurements made by the utricular and saccular otolith organs, indicating that the specific force vector now lies along the head y-axis, having rotated 90° from the head z-axis. Minor deviations from accurate determination of orientation associated with the A effect or with inaccuracies in canal transduction are ignored. The consistent interpretation based on canal and otolith signals is the veridical head roll. If this action were the result of active muscle contraction rather than passive rotation, the perception would furthermore be consistent with the efferent copy determination of the result of muscular contractions. Finally, if this action were performed with eyes open in a stationary environment, the perception would agree with that based on visual cues (relative rotation of the retinal image), taking into account the small effects of ocular torsion.

Simple examples of conflicting cue situations are more difficult to find, although one may be a common experience to the reader. During constant forward, x , acceleration lasting more than a few seconds, such as is experienced during the takeoff roll of an airplane, the specific force vector rotates rapidly from the vertical to a position lying in back of the true vertical. The passenger or pilot is forced back into his seat, pressure cues on the back increase, and the utricular otolith organ detects a component of specific force in the $-x$ direction. The new specific force vector lies between the x -axis and the $-x$ -axis, as shown in Figure

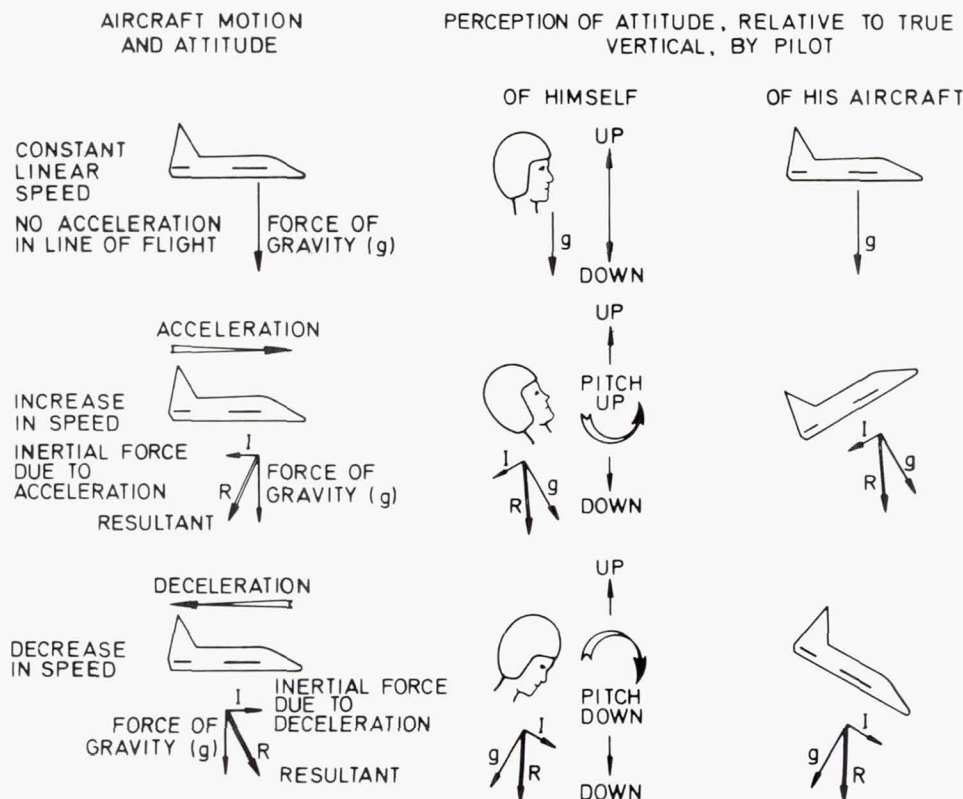


FIG. 20. Illustration of perception of pitch during constant acceleration. [From Benson (4a).]

20. All of the linear acceleration mechanisms therefore indicate a situation consistent with a change from the upright to a pitched-back orientation. The vertical semicircular canals, however, having received no angular acceleration input (neglecting the direct effects of linear acceleration on the cupulae for the moment), signal no change in pitch angular velocity, which was assumed to be zero at the outset. Consequently there is a conflict between the simple interpretation of linear acceleration cues as a pitch up and the lack of confirmation by semicircular canal signals. What in fact normally happens, in the absence of visual signals or a mental set dictating the contrary, is an initial perception of forward acceleration without any change in pitch angle, which only gradually shifts to the replacement of the acceleration sensation with one of steady-state pitching up. The generally accepted explanation is that, with the passage of sufficient time compared with the dominant time constants of the semicircular canals, the lack of a confirming semicircular canal signal is given less weight in the conflict with otolith signals, and that in the steady state the otolith signals dominate in perceiving static orientation with respect to the specific force vector. This pitching-up illusion is of practical importance when the accelerations are large. On catapult-assisted takeoffs from an aircraft carrier deck, the illusion of excessive pitching can be quite severe, even without consistent semicircular canal cues. The unfortunate pilot, who believes that he has been pitched up excessively during the launch and reacts by pushing the nose downward, risks an abrupt crash into the water. The following sections illustrate some of the better known examples of conflicting vestibular cues and their resolutions.

Centripetal Acceleration

Consider a subject who is riding in a fixed chair at the end of a centrifuge arm when the centrifuge rapidly spins up to a consistent angular velocity. If the subject is facing "into the wind" so that his y -axis lies along the centrifuge arm, the centripetal acceleration is directed inward along his $-y$ -axis (for counterclockwise centrifuge rotation) and the centrifugal force is directed out along his $+y$ -axis. The net specific force vector rotates from the true vertical outward, as illustrated in Figure 21. The specific force vector rotates outward by an angle θ , given by $\theta = \tan^{-1}(\omega^2/g)$. The subject senses the initial acceleration to angular velocity ω about his z -axis with his horizontal semicircular canals. After several canal time constants, the sensation of rotation about the z -axis decays to zero. The rotation of the specific force vector creates a situation similar to that of the pitch illusion during acceleration, discussed in *Ambiguity of Subjective Response to Acceleration*, p. 1046. Although the otolith cues and the tactile cues are all consistent with the simple explanations of a subject rolling outward by an angle θ with respect to the vertical, they are not confirmed by

signals from the vertical semicircular canals. Furthermore, if the subject had been actually rolling while yawing about his z -axis, cross-coupled angular acceleration would also have introduced a sensation of pitch, which is similarly lacking. In any event the perception that the vertical is aligned with the specific force vector lags dramatically behind the actual rotation of the specific force vector (67), as indicated in Figure 21. Presumably the delay in adopting the changing direction of the specific force vector as the vertical is tied to the conflict with the semicircular canal signals, and it awaits the passage of several time constants of the canals before these conflicting signals can be ignored.

Another situation in which the semicircular canal and otolith cues conflict is a coordinated turn in an aircraft. In this most common of all aircraft maneuvers, as illustrated in Figure 22, the aircraft is maneuvered from straight and level flight into a constant rate of turn ω by rolling to a bank angle ϕ . A typical pattern of rolling into and out of a sustained constant-rate coordinated turn of radius r is shown in the figure. The specific force vector, \mathbf{f} , which is the vector sum of gravity \mathbf{g} and negative acceleration $\mathbf{r}\omega^2$, remains

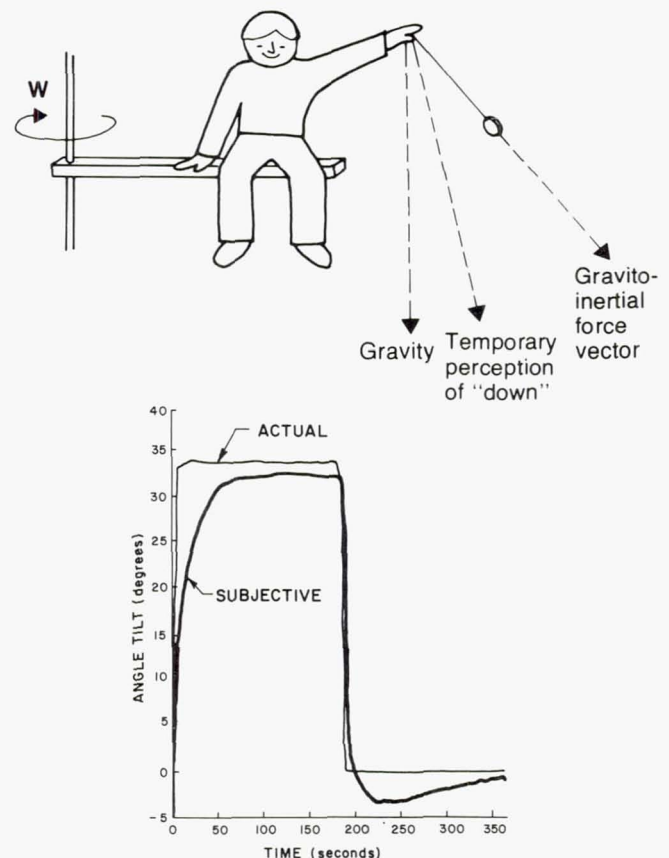


FIG. 21. Shift of direction of specific force vector during constant velocity centrifuge rotation produces slow shift in direction of estimated vertical or horizontal. [Adapted from Young (175a) and Graybiel and Brown (67).]

AIRCRAFT ORIENTATION

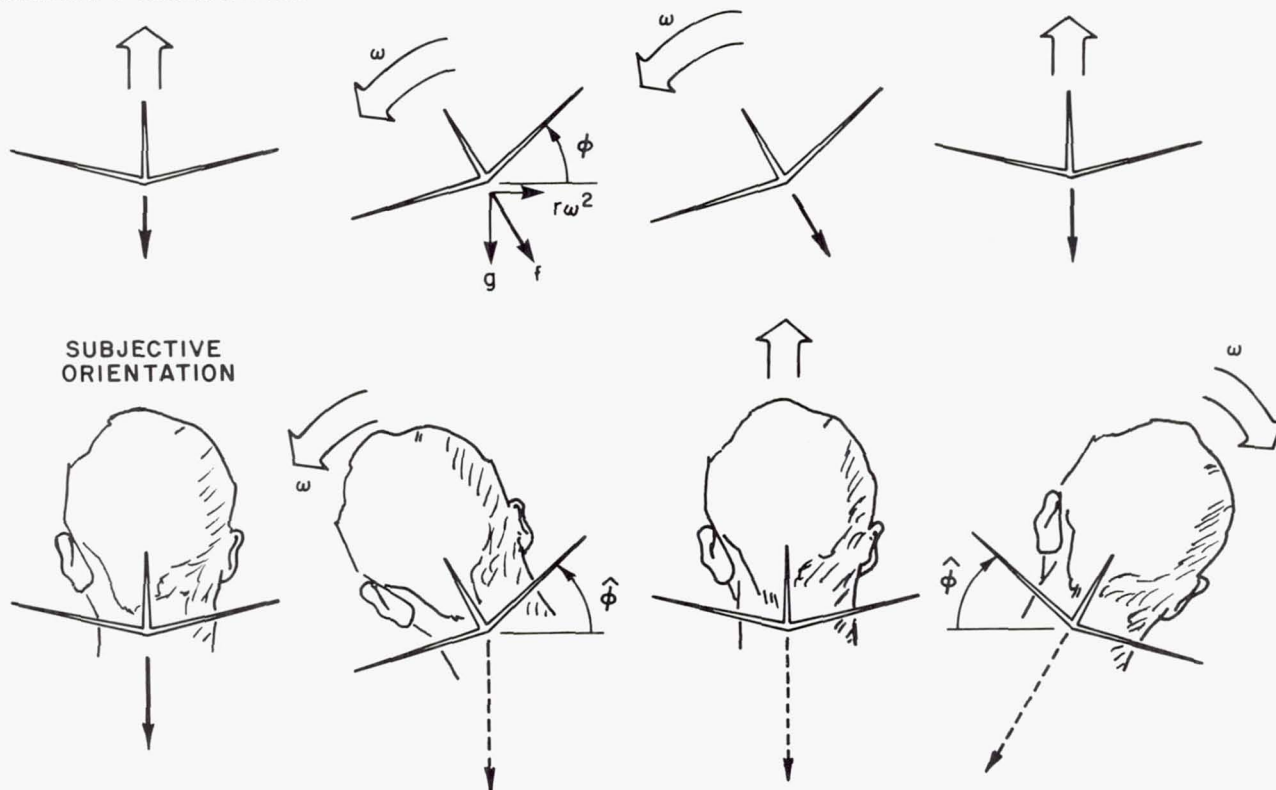


FIG. 22. Coordinated turn leading to error of spatial orientation.

aligned with the z -axis of the aircraft and of the pilot and passengers during the coordinated turn. As a consequence, a conflict develops between semicircular canal and otolith cues about the roll angle. The vertical semicircular canals, sensitive to the brief roll rate, correctly indicate the roll into the turn during initiation of the roll rate, and they indicate the existence of zero roll rate during the constant turn. The otolith system and the other graviceptor sensors, however, do not detect any change in the orientation of the specific force vector with respect to the body; the specific force vector always points directly down through the bottom of the aircraft. The only change the graviceptor sensors experience is a small increase in the magnitude of the specific force vector from g to $g(1 + r\omega^2/g)$. Because the graviceptive cues do not confirm the existence of a steady-state roll angle, the perception of this roll is quickly washed out, unless confirmed by visual cues, instruments, or previous knowledge of the maneuver. Furthermore, the turning rate ω is usually constant and becomes subthreshold. The perceived roll is indicated schematically by $\hat{\phi}$ in Figure 22. On rolling out of the coordinated turn and back into straight and level flight at the end of the maneuver, the reverse process takes place. Beginning with the perceived roll angle of zero, the subject interprets the transient semicircular canal cues as a roll in the opposite direction of the initial turn (to the right in Fig.

22). Because the direction of the specific force vector still remains aligned with the subject, however, this sensation of rolling in the opposite direction during straight and level flight is normally not maintained. The situation is further complicated by the postrotatory sensation in yaw (discussed in *Rotation in Dark*, p. 1029), in which the horizontal semicircular canals signal a postrotatory turn to the right, consistent with the illusion of roll to the right. The coordinated turn is a simple but well-documented case of practical importance in which the conflict exists between semicircular canal and otolith cues. It is resolved normally by short-term and transient reliance on semicircular canal signals (which are relatively high-frequency transducers of roll angle changes) and long-term or steady-state reliance on otolith and other graviceptive cues. It is of such practical importance in aviation safety that it serves as a fundamental case for teaching pilots during instrument flight to believe their instruments. In the presence of visual fields indicating the veridical motion of the aircraft, of course, the conflict is easily resolved correctly.

Cross-Coupled Angular Accelerations; Coriolis Illusion

A common perceptual illusion that causes almost as much confusion in its discussion as in its experience is

that of cross-coupling due to simultaneous rotation about more than one axis. Both the perception of spatial rotation and the discomfort associated with such head movements are explainable on the basis of signals processed by the semicircular canals and otoliths. Consider, first, a subject who is rotating at constant angular velocity counterclockwise (left to right) about his z -axis vertical, which is the normal position for spinning with the head erect. Shortly after beginning this rotation, the horizontal semicircular canals, having integrated the acceleration necessary to reach this velocity and not yet having had sufficient time for the cupulae to return, correctly indicate the magnitude and direction of his angular velocity. Let us call this velocity ω_z . Now permit the head to be tilted rapidly 90° to the left shoulder, assuming for simplicity a constant-roll angular velocity of ω_x for this tilt. During the tilting motion, the ongoing vertical-axis angular-velocity vector ω_z is rotated in head-fixed axes from the yaw axis to the pitch axis at an angular velocity of ω_x . This rotation, represented as an angular acceleration about the head roll axis, is a real angular acceleration of magnitude $\omega_z\omega_x$, directed along the y -axis. It would be detected by any angular accelerometer sensitive to accelerations about the y -axis, including the vertical semicircular canals, which detect pitch. Consequently the vertical canals detect and transmit the transitory acceleration leading to a perception of pitch forward during the period of head tilting. (This transient acceleration disappears when the head has reached its new position.) As a result of this cross-coupled acceleration, the ongoing angular velocity about the vertical axis ω_z has been removed as a stimulus from the yaw semicircular canals and appears as a stimulus to the vertical pitch semicircular canals. The horizontal canals signal a deceleration to zero, and the vertical canals signal an acceleration to the actual vertical axis angular velocity, which is now about the head pitch, x , axis. All of these phenomena represent the veridical situation and, to this point in the discussion, would cause no conflicting signals or disorientation. In fact, such a head movement carried out just after initial acceleration causes minimal disorientation and no discomfort (77).

The more interesting and more disturbing aspects of cross-coupled angular acceleration occur when the head tilt is made after a prolonged period of constant angular velocity. If we assume that the angular velocity of spin about the vertical axis discussed in the paragraph above has been going on for 30 s or more prior to the head tilt, then it is apparent that prior to the head tilt the yaw semicircular canal cupula would have returned to its rest position and the perception of angular velocity about the z -axis would also have returned to zero, as illustrated in the left portion of Figure 23. Assuming that the roll rotation is a passive one in the dark, the subject would be taken completely by surprise by the resulting yaw and pitch semicircular canal signals and the perceptions that ensue from a

head tilt to the left shoulder during this ongoing spin. The transient cross-coupled angular acceleration indicating a pitch-back sensation during head tilt occurs, just as in the case discussed above. In this situation, however, because the subject would presumably have an indication of ongoing z -axis angular velocity, the sensation would come as a complete surprise and does, in fact, cause alarm and confusion. The pitch axis semicircular canals are accelerated from null to ω_z and indicate to the subject a sudden and unexpected acceleration about the vertical axis ω_z , as above. The horizontal semicircular canals, having been in their rest condition prior to the head tilt, indicate a sudden deceleration when the ongoing vertical axis counterclockwise velocity is removed from them by the head tilt. The horizontal semicircular canal cupulae are deflected by this deceleration, and they indicate an opposite (postrotatory) velocity, this time in the clockwise direction about the head yaw axis, represented by the yaw component of ω_z . Consequently, following the cross coupling associated with the head tilt (which would be detected even by perfect angular-velocity detectors), this head movement leaves as an unpleasant residue a sensation of spinning clockwise about the yaw axis, which is now in the horizontal plane. This aspect of the cross coupling, which is certainly unexpected and unpleasant, depends on the imperfect integration in the semicircular canals. To make matters worse, there is a discordance between the signals indicated by the semicircular canals and those indicated by the otoliths. For the first case discussed above, in which the head movement is made immediately at the initiation of the vertical axis spin, the otoliths signal the veridical head orientation with respect to vertical, and the only conflict comes about during the head tilt itself, when otolith signals do not indicate any pitch. For the second case, in which the horizontal semicircular canal deceleration results in a perception of clockwise rotation about the yaw axis horizontal, there is an absence of any otolith time-varying signal to confirm this sense. Stated more simply, the horizontal semicircular canals signal a continuous rotation, but the utricular and saccular maculae indicate that the head has not, in fact, succeeded in changing its orientation with respect to gravity. The illusions of pitch and roll, when considered in detail, are relatively complex (77, 113). The sensations of discomfort and frequently of motion sickness resulting from these cross-coupled angular accelerations are most likely attributable to the conflict between semicircular canals and nonconfirming otolith cues rather than to the unexpected cross-coupled angular acceleration signal acting on the canals themselves. Some support for this theory stems from the results of the cross-coupled angular-acceleration experiments carried out during weightlessness on the Skylab space mission. Although the initial tests were not carried out early during the weightless period, so the possibility of generalized motion sickness habituation exists, it

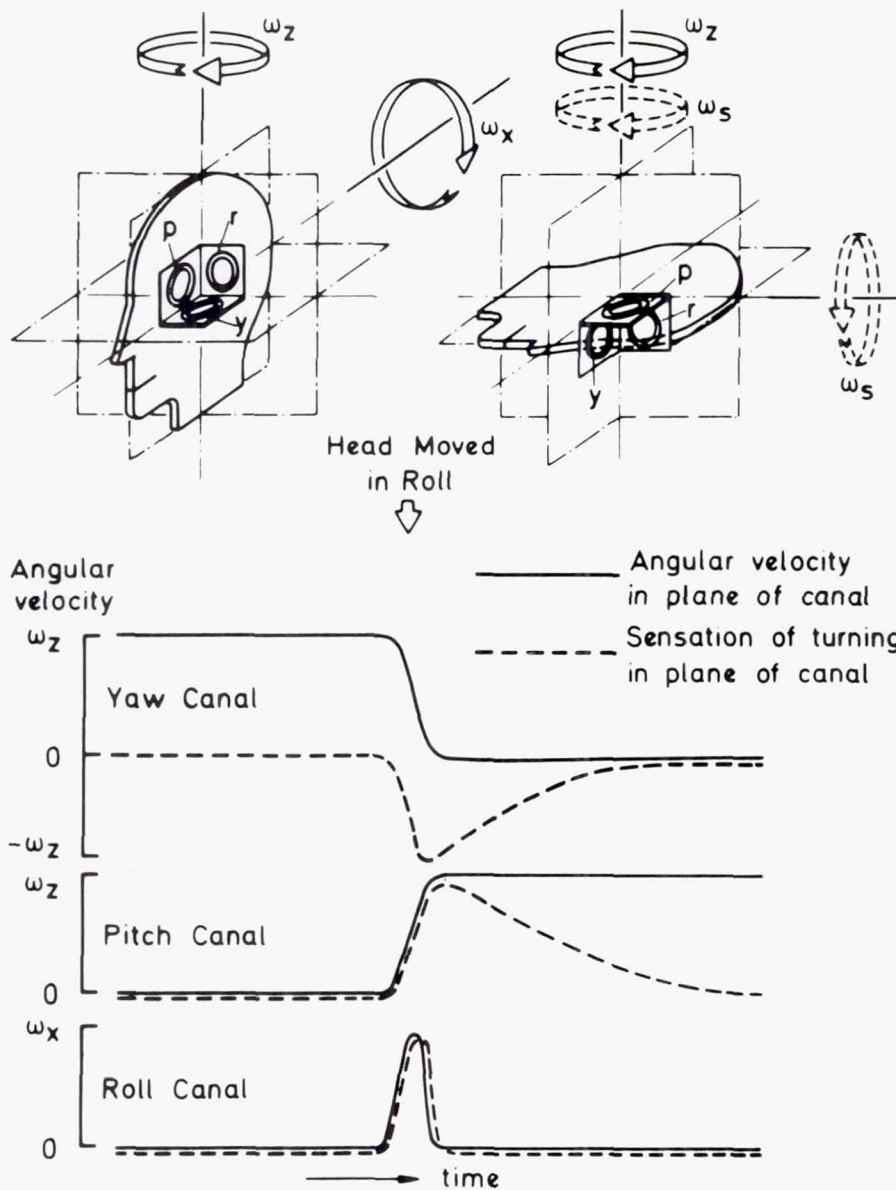


FIG. 23. Illustration of cross-coupled stimulation. Rolling head movement, ω_x , during sustained yaw rotation, ω_z , leads to erroneous transient perception of yaw and pitch velocity. [From Benson (4a).]

was found that subjects were able to make essentially unlimited numbers of cross-coupled angular-acceleration head movements in the weightless condition without reported motion sickness symptoms, such as they reported under similar conditions on the ground. In weightlessness, of course, no steady-state otolith conflict existed to support or to contradict the semicircular canal cues following head movements (70).

Rotation About Off-Vertical Axes

It was indicated in *Semicircular Canals*, p. 1025, and *Otolith Organs*, p. 1026, that the principal role of the semicircular canals in perception of orientation is

detecting angular velocity, whereas that of the otolith organs is detecting either static orientation with respect to the vertical or linear acceleration. Changes in the otolith signal that are interpreted as changing orientation with respect to the vertical may also be used to infer angular velocity, in which case their information is comparable to that normally expected from the semicircular canals. The simple rolling of the head to the shoulder is an elementary example of this multiple-cue integration. A more complex example, which does not normally occur in daily life, involves sustained constant-angular-velocity rotation about the longitudinal z -axis when this axis is off vertical or not aligned with gravity. Consider for simplicity the

situation in which the longitudinal axis is aligned with the earth horizontal and rotation is carried out at constant angular velocity about the yaw axis. This bizarre orientation is sometimes referred to as "barbecue spit stimulation." On initiation of the rotation, of course, angular-velocity signals from the vertical (roll) semicircular canals are in agreement with the changing orientation of the head with respect to gravity, indicated by the otoliths. After 15-30 s of constant-angular-velocity rotation, however, the semicircular canal signals have decayed toward zero, except for a small remaining gravity-sensitive modulation. Nevertheless the perception of the actual continuous rotation remains during the stimulation (4, 6, 35, 76). Clearly this is a case where there is a conflict between semicircular canal information, which decays to its rest state and does not indicate any rotation (although strictly speaking it supports any constant-velocity hypothesis) and dynamic otolith information, which is consistent with continuous rotation. Otolith information in these instances appears to dominate. Simultaneous recordings of nystagmic eye movements (4) and the results of experiments with selected blockage of semicircular canal or otolith signals support the view that otolith information is processed to indicate the sustained rotation. A closely related conflict occurs when the subject is brought to a sudden stop after barbecue spit rotation, and the previous rotation axis remains in its off-vertical orientation. The postrotatory sensation signals from the semicircular canals, opposite in direction to the per-rotatory motion, are in conflict with the signals from the otolith organs that indicate static orientation with respect to the vertical. No significant postrotatory turning sensation occurs, and the postrotatory nystagmus is much weaker and briefer than for rotation about a vertical axis. Evidently when otolith and tactile cues or previous knowledge of the stimulus situation indicate to the subject that any motion will be about an off-vertical axis, confirming graviceptor cues are necessary and sufficient to produce the perception of rotation or inhibit it, despite transient semicircular canal signals. Similar perception of constant angular velocity of pitching about the pitch axis occurs when the subject is rotated at constant angular velocity with the y-axis horizontal. [One report, however, based on a single subject rotated at constant angular velocity with tactile cues minimized by underwater immersion, contradicted this observation and described a perception of circular or elliptical counterrotation as if on a ferris wheel (151)]. Guedry (75) demonstrated that an intact vestibular apparatus is required both for the development of the continuous rotation sensation during off-vertical rotation and for the associated nystagmus. The absence of these responses in subjects lacking a normal vestibular apparatus (75) is strong evidence for vestibular contributions to the sustained rotation sensation. The possibility of direct linear acceleration effects on the semicircular canals is still open, although the bulk

of evidence supports the central integration of otolith and canal signals (4).

VISUAL EFFECTS ON PERCEIVED ORIENTATION—MODELS

Static Visual Orientation

Straight lines in a visual field play an important role in defining a subject's perception of the vertical or horizontal. When a subject looks at a tilted room, or one that is made to appear tilted through the use of prisms or mirrors, he associates the vertical with the line tilted toward the direction of the walls of the room. If the subject is placed in such a room, his postural reactions are consequently to align himself parallel to the walls, and his reactions are consequently destabilizing. The tendency to associate trees, walls, and the like with the vertical and apparent horizons with the horizontal is at the basis of many well-known illusions, such as the "streams that run uphill" or the "haunted swing" (172). Serious disorientation of pilots can result from the illusion that the tops of a tilted cloud bank or the line of street lights near the edge of a town represent the horizon (60, 61). The extent to which visual scene straight lines influence the perception of vertical or horizontal is highly variable among individuals. The field dependence or field independence is generally measured by use of the "rod and frame" test (171), in which the subject is required to align an illuminated rod to the perceived vertical when the rod is surrounded by a large square frame which may be tilted from an upright position. Although individual differences in field dependence have been associated with sex, intelligence, and personality traits (170), the basis for the field dependence is not fully understood.

Meaningful elements in static visual scenes are not essential to influence the perception of the vertical. Any distinct axes, which may be contained in a pattern of parallel stripes or on a grid, produce a tendency to align the closest vertical or horizontal with the axis (60, 142).

Moving Visual Fields

Uniform motion of a large part of the visual field can induce a false sensation or illusion of self-motion. A common example occurs to the passenger in an automobile or train, himself stationary, when the neighboring vehicle begins to move slowly. The common illusion is that one's own vehicle is moving in the opposite direction. Such visually induced self-motion, in which the relative movement of the visual field is perceived as attributable partially or entirely to movement of the observer through the field, is known asvection. It is used to advantage in the induction of a sense of motion in wide-field-of-view flight simulators, large-screen movies, and in various amusement park

exhibits or rides in which the sensation of movement is created or heightened by visual surround motion.

When a wide visual field suddenly begins to rotate about a stationary subject, the perception of veridical-field motion is generally replaced by a perception that the visual field slows, often to a stop, and is replaced by subjective rotation (circularvection) beginning typically 2–5 s after stimulus initiation for rotations about a vertical axis. The strength of the self-motion sensation builds gradually over a period of 3–10 s, rising in a roughly exponential fashion to some steady level. For field velocities less than 50 deg/s, the circularvection may be complete or saturated, in which case the visual field is perceived as stationary in space. If the onset of the visual-field motion is gradual, at acceleration levels comparable to the acceleration thresholds of the semicircular canals (on the order of 0.2 deg/s), then the latency to onset of circularvection may be negligible, and all of the perceived motion may be interpreted as self-motion. A number of factors contribute to the strength of vection and its rapidity of onset.

The peripheral visual field is of primary importance for the development of vection (72). Although the moving visual field generally induces nystagmic eye movements beating in the direction of the field, such eye movements are not required for generating self-motion. In fact, oppositely beating nystagmus may be generated by a central moving field without interfering with the peripheral field-generated vection (19, 54). Although the specific content of the moving visual field is not particularly important for inducing vection, the spatial frequency of the field and in particular the rate at which contrast borders move over the retina is important in generating vection effects (84). Moving objects that appear to be in the background of the visual scene are considerably more effective in generating vection than are moving objects in the foreground.

The subject's view of his own body can only enhance vection, whereas visible fixed objects in the background interfere with it (19). The relationships between the characteristics of the visual field and the dynamics of vection are covered in several recent reviews (44, 45, 86, 175). Although the quality of the visually induced motion is in almost all respects identical to the motion perception resulting from true body motion with its attendant vestibular and proprioceptive cues, there exists at least one important difference. Vection exhibits relatively frequent and not yet explained dropouts, in which the sensation of self-motion is suddenly reduced to zero and replaced by the veridical sensation of visual-field motion. During these dropouts, although the sensation of velocity changes, there is no simultaneous sensation of sudden deceleration. The relationship of these dropouts to eye movements and conflicting vestibular signals is still being explored. Furthermore, during exposure to continuous constant-velocity visual scenes, the visually induced

motion sensation gradually adapts so that the perceived self-velocity during sustained constant-field velocity stimulus is gradually reduced (8). A common example of linearvection adaptation is the underestimation of automobile speed after prolonged high-speed driving.

In addition to the continuous perceived self-motion velocity, a paradoxical self-motion sensation is created when viewing a field that rotates about the axis of gaze along an earth horizontal axis. This illusion, termed visually induced tilt (46), may easily be observed by staring at the axis of a disc rotating with its axle horizontal. The initial illusion resembles that of circularvection, a perception that the field slows to a stop and that the viewer begins to fall in the opposite direction. This self-rotation sensation, however, does not result in a continuous and ever-increasing perception of roll or pitch angle, which would lead to a perception of inversion and beyond. Instead, a static perception of tilt ensues in the direction opposite to that of visual-field rotation, leading to inappropriate postural responses and to the setting of the perceived vertical in the direction of field motion. The paradoxical self-motion perception (continuous rotation without increasing angular deviation) is apparently created by the conflict of visual signals with the otolith sensory information that indicates no tilt about the horizontal axis.

Static Visual-Vestibular Interaction

The retinal signal (proximal input) from a fixed external visual field (distal input) varies with head tilt. This variation of proximal signal must in some manner be compensated for head and eye movements in order to have vertical objects continue to appear vertical despite head tilt. As discussed in *Static Orientation to Vertical*, p. 1039, this compensation is not perfect but results in systematic underestimation or overestimation of tilt (the Aubert and Müller effects). There clearly exists some mechanism by which varying retinal images lead to perceptions of the same orientation of the visual distal signal. As illustrated schematically by Bischoff (9) in Figure 24, the interference variable associated with head tilt is presumed to be compensated. Although there is some measure of external compensation consisting of ocular counterrolling, in which the torsional movements of the eye are in the direction necessary to maintain ocular stability despite head movements, these are vestigial in man, and the tonic steady-state ocular torsion for head movements up to 60° is normally less than 10% of that required for full compensation. Consequently one must look to some internal process for the compensation of the tilted retinal signal as a result of varying head roll angles. The most straightforward of the internal compensation mechanisms, building on early outflow ideas of Helmholtz (85), Bühler (23), Kardos (95, 96), and others, was later formulated by Von Holst and Mittel-

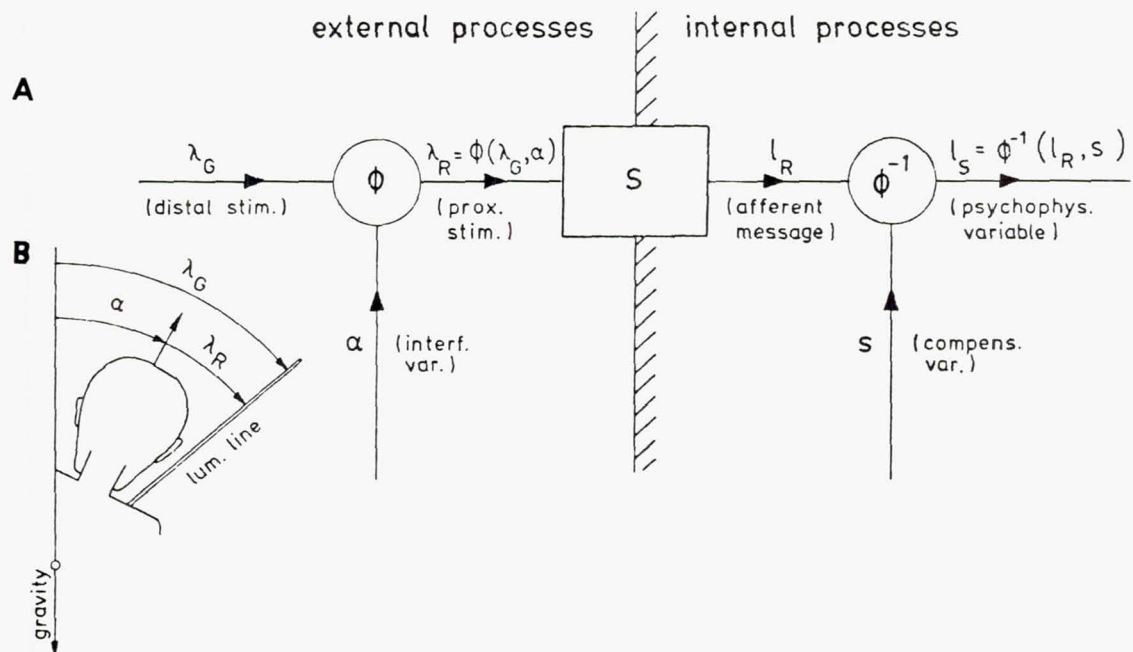


FIG. 24. Schematic representation of compensation principle for effect of head tilt on retinal image or proximal stimulus. ϕ , Interference function; ϕ^{-1} , compensatory function; S, mapping function of sensory channel. [Adapted from Bischoff (9).]

staedt (162) and others as the refference principle. When applied to the correction or reinterpretation of the visual image by nonvisual signals, indicating head tilt, this compensation is referred to by Bischoff as heterocompensation. Simply stated, vestibular, kinesthetic, or tactual cues assess the amount of head tilt and apply the appropriate internal correction signal to compensate the tilted visual scene and allow it to be perceived as upright. To complicate the story, however, are the observations that the orientation of the visual field itself can lead to changed perception of the vertical, which in turn can affect the perception of the orientation of that scene. Specifically, the indication of the vertical is influenced by the orientation of a background visual scene containing axes that may be thought of as vertical or horizontal, by head orientation, and by the interaction between these two stimuli. Based on a complex set of experiments involving rotations of both the head and the visual field about a vertical axis, Bischoff and Scheerer (10) arrive at evidence to support the feedback-autocompensation theory for the manner in which the compensation signal is developed based on optical as well as vestibular inputs. The perceptual output itself is involved in the filtering process that goes into determining the sense of vertical, and this axis rotation then feeds back to participate in interpretation of the proximal image coming from the retina. Because visual as well as vestibular cues influence the perception of self-orientation and consequently the interpretation of the orientation of visual scenes, as well as postural reactions, the interaction of static visual and vestibular

signals provides an interesting challenge to mechanistic modeling. Referring primarily to evidence from the static orientation of fish in response to varying angles of incident light, Von Holst and Mittelstaedt (162) expressed the relative influences of the two sensory inputs on orientation in terms of variable weighting functions. They (162) formally developed a trigonometric addition theory for this linear weighting notion. Even in fish, as Von Holst pointed out, the relative spatial orientation weighting given to optic input depends on how interesting or meaningful the object is in the visual scene.

In dealing with human spatial orientation problems, especially in the context of disorientation and motion sickness, many investigators stress the importance of intersensory conflict and its resolution. Young (174) represented the visual-vestibular interaction problem in humans as a flow chart (shown in Fig. 25) in which visual and vestibular responses to orientation were weighted linearly provided they compared reasonably well, reminiscent of the ideas of Von Holst for fish orientation. When the deviation between the visual and the vestibular signals exceeded some tolerable level, however, the content and quality of the visual information was examined to determine whether or not it was "compelling." A compelling visual field, consisting of recognizable objects and strong orientation cues, would tend to then be accepted as representing the external vertical reference, whereas uncertainty in accepting the visual field would lead to greater reliance on the otolith signals. If, however, neither were compelling (as for example in the head-

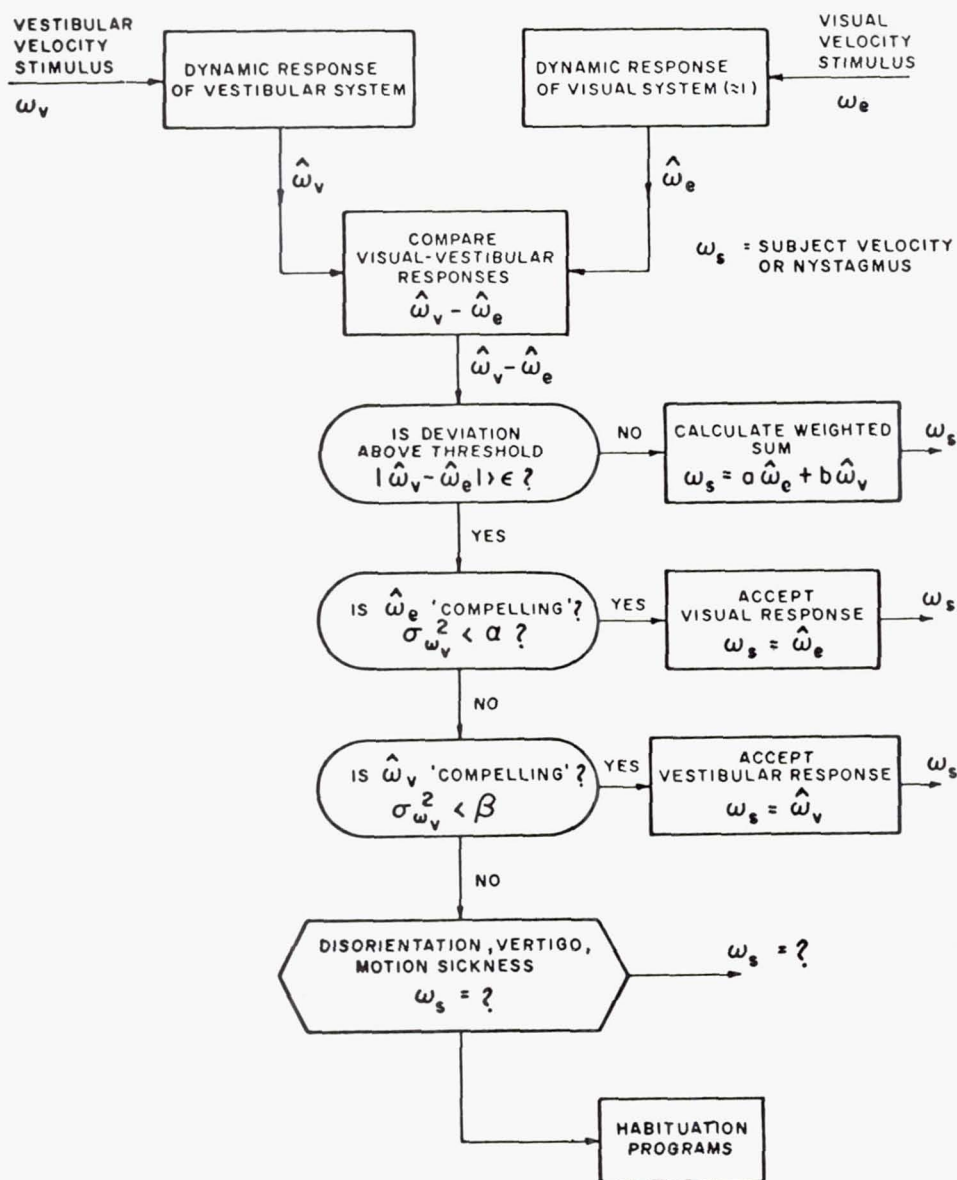


FIG. 25. Flow-chart representation of visual-vestibular interaction. [From Young (174).]

inverted situation), then disorientation, vertigo, and motion sickness might be expected to result.

Bischoff and Scheerer (10), in attempting to model visual-vestibular interactions for relative influences of visual fields in different head orientations, proposed a model that incorporated both the notion of linear weighting of visual and vestibular cues and also the idea that visual cues would be given greater weight in situations in which the otolith cues were associated with a high uncertainty. [It had already been demonstrated by Udo de Haes and Schöne (158) that the strength of visual orientation cues increased as head tilt varied from the erect position, and the strength of these cues generally followed the variability of the orientation perception based on otolith cues alone.] The systems analytic model of optical vestibular in-

teraction in determining the vertical developed by Bischoff and Scheerer (10) and discussed by Bischoff (9) matches the perceived vertical data rather well. A somewhat modified version of this model is given in Figure 26. In this model, the compensatory signal C, which corrects the tilted retinal image of the target angle for head tilt, is generated on the basis of both feed-forward compensation from otolith cues regarding head tilt and feedback autocompensation based on the content of the image itself. The angle of the retinal image R would be equal to the target angle T except for the influence of head tilt H, which is only partially compensated physically by ocular countertorsion E. The otolith system is shown to generate ocular torsion in a compensatory manner, and the sketch of E versus H is meant to imply that ocular torsion peaks at about

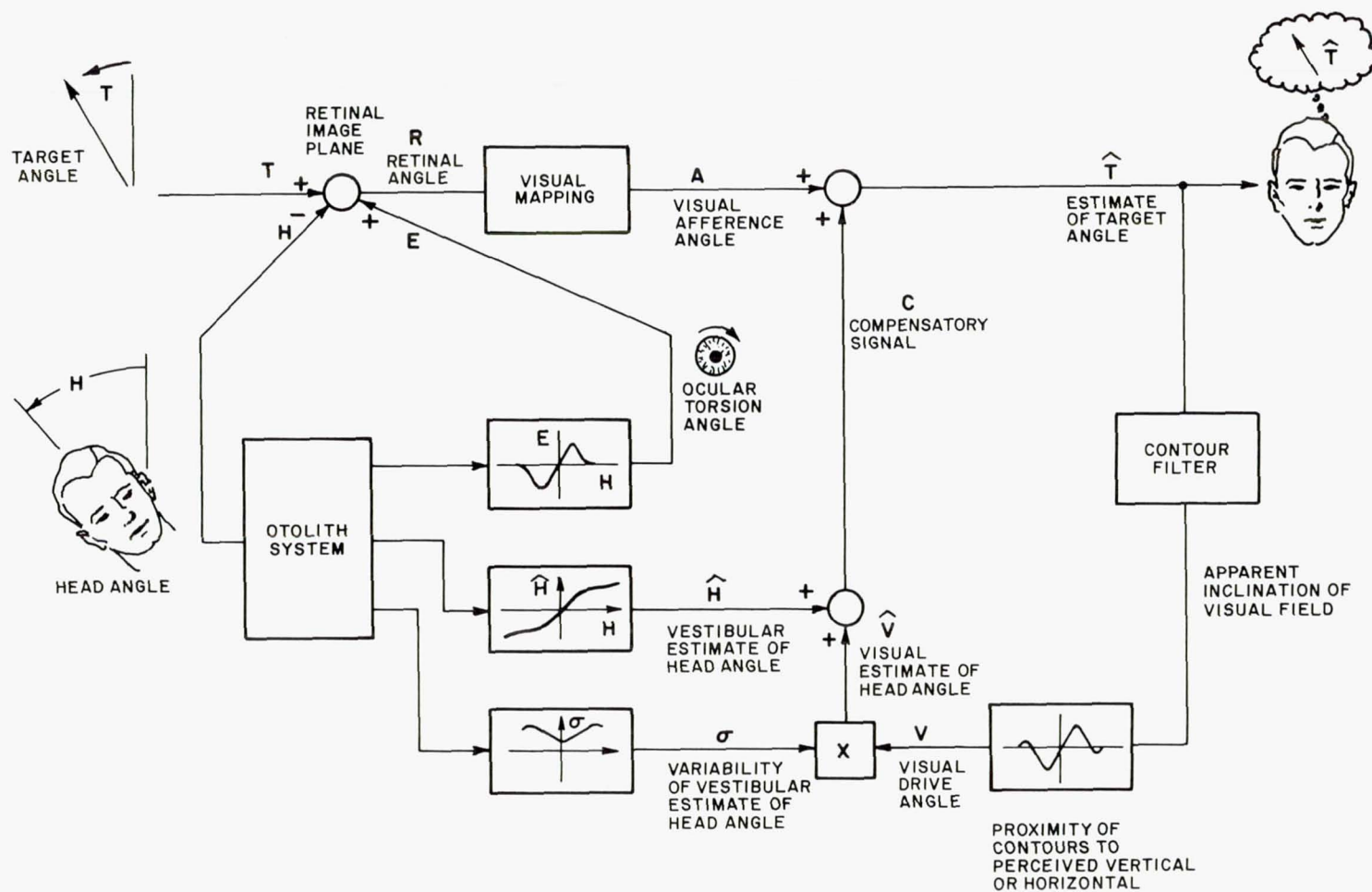


FIG. 26. Model of optic-vestibular orientation to the vertical. [Adapted from Bischoff (9).]

60° rather than at 90°, as would be predicted on the basis of lateral shear force on the utricular macula alone. It should be noted that no provision is made for ocular torsion to be influenced by the visual scene or the perceived head tilt angle, although these effects probably do occur.

The retinal angle R is mapped to a cortical representation based on the visual afference A , which must be corrected for the effects of H and E by a compensatory signal C ; the C is presumed to be the result of a linear weighting of the vestibular drive \hat{H} and the visual drive \hat{V} . The vestibular portion of the compensatory signal is developed on the basis of otolith cues. The curve of \hat{H} versus H is meant to indicate the slight overestimation of tilt at small angles and the underestimation of tilt at larger angles of head tilt or the Müller and Aubert effects. The feedback autocompensation portion of this model assumes that the final estimate of target angle, which represents the perception of the target orientation in space, is passed through a contour filter to yield a signal representing the apparent inclination of the visual field with respect to the perceived vertical or horizontal. When the dominant axes lie close to the vertical or horizontal, they are known to influence static orientation perception by pulling the perceived vertical toward the seen axis, and this is shown in the block "proximity of contours to perceived vertical or horizontal." The resulting visual drive angle V is multiplied by σ , which represents the variability of the vestibular estimate of head angle. For the head erect, in which the vestibular signal has low variance and in which accurate estimates are made, the visual angle is consequently given little weight, whereas for the head tilted far from the vertical, σ is larger and the visual weighting is greater, resulting in a larger signal \hat{V} . Finally \hat{V} , the visual estimate of head angle, is added linearly to H to produce C .

Although this model has not apparently been tested for a wide variety of hypergravity and subgravity tests, nor is it meant to represent anatomical locations of the functions, it serves as a useful quantitative statement of the autocompensatory feedback notion combined with the reafference principle for compensating head angle.

Vection and Dynamic Visual-Vestibular Interaction

When a subject is moved actively or passively in conjunction with exposure to moving visual scenes, the resulting visual-vestibular interaction provides a rich test of the capabilities of the human spatial orientation system for dealing with normal and abnormal multisensory integration. Visual cues normally confirm the orientation information conveyed by the vestibular, proprioceptive, tactile, and motor command copy information. Thus, for example, when a subject tilts his head toward his right shoulder, the image of the stationary visual field on his retina rotates as

though the external visual field were moved counter-clockwise, his semicircular canals signal a transitory clockwise angular velocity that is integrated to yield an estimate of change in head angle and incidentally some compensatory eye torsion. Otolith signals register a new head orientation with respect to the vertical, confirming the semicircular canal and visual cues. Neck-joint angle receptors and possibly neck muscle spindles confirm the new head position and support the expectation, based on the internal model concept, that the commanded motor program was indeed fully affected. This represents a clear case of confirming visual and vestibular signals, leading to an unambiguous perception of spatial orientation. Similar comments apply to simple left-right head movements.

A wide variety of interesting and nontrivial conflicting visual-vestibular interaction cases may also be considered, although they have little to do with normal function. The normal relationship between head movement and relative movement of the visual field may be disturbed in a number of ways. When a subject wears left-right reversing prisms, field rotating prisms, magnifying or minifying spectacles (including reading glasses), the normal relationship between vestibular signals and visual signals on head rotation is disturbed. Rotation of the head under increased gravitational forces produces otolith signals that differ from the visual and semicircular canal representations of head movement. Head rotation in zero gravity fails to produce the normally expected tonic change in otolith activity to confirm the head movement. Nodding the head about an axis normal to that of continuing rotation produces the cross-coupled angular acceleration phenomenon discussed in *Cross-Coupled Angular Acceleration; Coriolis Illusion*, p. 1051, and creates motion illusions that conflict with the observed visual-field change. Visually induced self-motion in a flight simulator, which is not confirmed or only partially confirmed by concomitant vestibular cues, is another example of conflicting visual-vestibular interaction. Because the visual and vestibular inputs to motion perception can be easily controlled and because the perceptual and motor output (ocular stabilization and posture control) are relatively easily measured, visual-vestibular interaction has been extensively studied as a paradigm for investigation of the more general question of multisensory integration (45, 86). The discussion that follows summarizes only a few of the more striking and unambiguous observations about the interaction between vestibular stimulation and motion sensation based on moving visual scenes.

The extent of visually induced tilt, either in pitch or roll, is limited by graviceptive cues that may not confirm this tilt. Thus the paradoxical sensation of rotation about an earth horizontal axis with only a limited illusion of induced tilt is attributable to the otolith signals. These, as well as other graviceptive systems, are presumably issuing conflicting commands indicating that head orientation is not changing rela-

tive to the vertical. The central nervous system possibly compromises its orientation computation between a visual signal of ever-increasing tilt and a graviceptive signal indicating no tilt, and it arrives at some intermediate position. This phenomenon is analogous to the case described above for static field influence on the perceived direction of the vertical. When the visual field rotation is about a vertical axis, with the subject lying supine, the perception of rotation continues unabated with no limitation on the visually induced roll angle. Here, clearly, the otolith signals provide no information that confirms or denies the visually induced tilt. An even more powerful demonstration is the effectiveness of a rotating wide visual field on the onset and strength of continuousvection in weightless conditions, where no relevant otolith signals are present to confirm or deny the visual input (177). Intermediate head orientations, corresponding to positions in which estimates of the vertical based on graviceptive cues have high variance, showed stronger visually induced tilt (179), just as they showed stronger static visual scene influences (10, 157, 158). All of these findings strengthen the view that visually induced tilt is limited by the lack of confirming otolith signals and that, when no such confirmation is to be expected (as in the case of zero gravity), the extent of the visual effect is not limited.

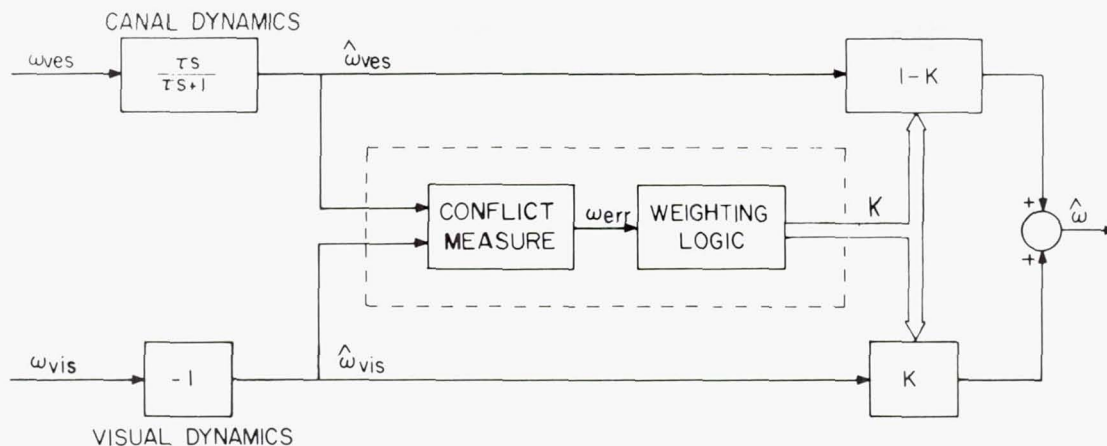
Similar limitations of the lack of confirming vestibular cues on visually induced motion are seen in yaw, where rotating visual fields induce circularvection. The delay in onset of circularvection and its gradual buildup is presumably the result of resolution of a conflict between visual motion signals and the absence of confirming semicircular canal cues. Visual-field acceleration at low levels, commensurate with semicircular canal signals close to threshold, do not show measurable latency, and even rapid visual scene acceleration can lead to immediate sensations of self-motion if a small true vestibular cue is generated by slight body motion in the confirming direction. Conversely, the presence of an established circularvection can mask the detection of small body motions in the opposite direction and bias the perception of angular acceleration based on suprathreshold vestibular stimuli (176, 180). Finally, the relative domains of influence of visual and vestibular cues are separable and correspond to the frequency ranges in which each is a reliable transducer of self-motion. Continuous perception of velocity is supported by the low-frequency relative motion of the visual field, whereas vestibular cues, especially those from the semicircular canals, are adapted out and contribute relatively little low-frequency information. At high frequencies, on the other hand, reliance is much more upon vestibular than visual cues, especially when the two are in conflict. The rough division between high and low frequencies for this case is generally thought to lie in the vicinity of 0.1 Hz.

A number of theoretical models for visual-vestibular interaction or for multisensory integration have been

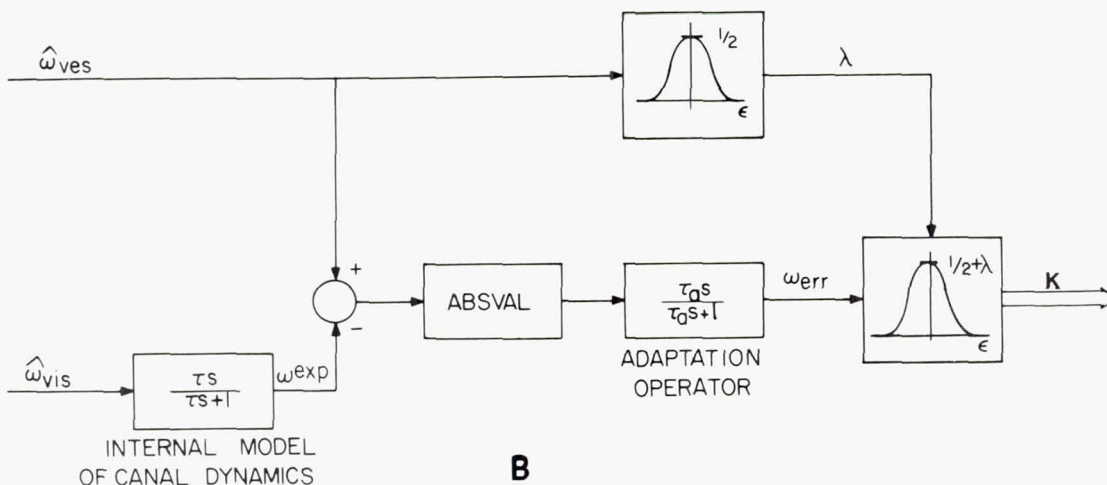
proposed (86). One of these compensation schemes, tested for static tilt of body and scene, was described in *Static Visual-Vestibular Interaction*, p. 1055. A more general notion, which has been discussed in a nonmathematical manner by many authors (e.g., refs. 76, 144, 174) is based on resolution of the conflict between visual and vestibular cues that results in changing the relative weighting applied to each of these sensory modalities. The weighting for each channel depends on the dynamic characteristics of all sensory channels and the amount of intersensory conflict. A mathematical treatment of the sensory conflict theory for spatial orientation that has been tested extensively for rotation about the vertical axis is presented in Figure 27 (180). In this model the gain, K , which controls the relative weighting of the visual system compared to the vestibular output, is adjusted according to a measure of cue conflict (ω_{err}). When the conflict between visual and vestibular signals is high, the relative weighting given to the vestibular input increases as K is reduced. In calculating the cue conflict, the difference is taken between the current vestibular signals, processed by the semicircular canals and indicating the vestibular system's estimate of angular velocity ω_{ves} and an appropriately filtered visual signal ω_{vis} . This visual signal is arrived at by passing the visual-field angular velocity through a first-order filter, which represents an internal model of the vestibular dynamics, to yield ω_{vis} . This signal corresponds to an internal representation of what the semicircular canal signals would be if the actual visual-field velocity were representative of head motion in a stationary field. The effect of this cue conflict adjustment of weighting function, which makes the model nonlinear, is to shift the weighting of sensory signals away from vestibular when the vestibular signals would either be highly variable or unlikely to present meaningful information about the relatively slow changes in true body velocity. On the other hand, where sudden changes in visual-field velocity are not borne out by actual vestibular signals on the basis of visual cues, then the visually induced motion is largely ignored in favor of reliance on the vestibular cues. Although this specific cue conflict model implementation has been developed primarily for modeling visual-vestibular interaction in rotation about a vertical axis, the concept of optimal mixing of multiple sensory cues appears to be generally valid and has been extended to other areas as well (15).

SPATIAL ORIENTATION IN ALTERED ENVIRONMENTS

Many of the illusions of spatial orientation associated with unusual or incompatible visual and vestibular stimuli are explainable on the basis of the conventional processing of sensory data. When these unusual sensory combinations persist for an extended period of time, from hours to days, the very meaning of the sensory signals in terms of spatial orientation is altered



A



B

FIG. 27. Sensory conflict model for resolution of visual-vestibular interaction. A: dual input conflict model; B: conflict measure and weighting function. [From Zacharias and Young (180).]

and so is the manner in which perception takes place. The remarkable plasticity of animals to adapt to altered environments by altering sensorimotor functional pathways is particularly evident in the plasticity of the vestibuloocular reflex. Human spatial orientation shows a similar plasticity that allows one to adapt both orientation perception and posture control to various altered environments. An outstanding example of this plasticity is the adaptation of orientation to weightlessness after several days of space flight. The process of resolving the sensory rearrangement involves the formation of new internal models. This process is closely related to the topic of motion sickness, which occurs principally in unusual environments and in unexpected combinations of sensory stimuli. It is believed that the process of adaptation to altered spatial environments is closely linked to the process of overcoming motion sickness.

Motion Sickness

Humans and most animals can be made motion sick

by exposure to a wide variety of motion and visual stimuli, provided the vestibular system is intact. Generally speaking, motion sickness occurs only under stimulus conditions associated with man-made vehicles and does not occur during motions typical of the normal range of natural head movements. A large number of fanciful theories for motion sickness and the remedies suggested by these theories are discussed in the excellent book on this subject by Reason and Brand (135). No satisfactory general theory for the survival value of motion sickness symptoms exists (154). It is by no means clear why motion sickness should in any way be tied to an evolutionary process, since it is a disorder resulting only from the use of technology. The neural pathways involved in motion sickness genesis are only slowly being worked out (66, 93, 120).

The most generally accepted current theory of motion sickness genesis is the conflict hypothesis. According to this theory, motion sickness is the result of conflict between spatial orientation signals coming from two or more different sources (76, 125, 135, 144).

These conflicting signals may be within a sensory modality (semicircular canals versus otolith) or between sensory systems (visual versus vestibular). The conflict might also be between sensory signals and the anticipated motion based on motor output or between sensory information and the expected pattern of such signals based on previous experience or continuing motion. (As a simple example of the last case, consider the unusual vegetative response when an elevator begins to move in the unanticipated direction.) Air-sickness, car sickness, and seasickness involve both intravestibular and visual-vestibular conflicts. The intravestibular conflicts arise from sustained linear or angular velocities at frequencies below the normal range of the semicircular canals or otoliths, particularly for airsickness. If the visual reference frame of the cabin or automobile is assumed inertially fixed, it is in conflict with the motion cues received from the otoliths and semicircular canals. Concentration on a visual task inside the cabin, such as reading, worsens the situation, whereas reference to an outside visual reference such as the roadway or horizon lessens the conflict and the symptoms if preventive action is taken early enough. (The reason that it is easy to read road signs but difficult to read a newspaper inside a bouncing bus is that the vestibular apparatus attempts to maintain ocular stability with respect to the outside visual field. This vestibular reaction must be suppressed and overridden by conflicting visuomotor reactions to permit concentration on the newspaper.)

Space motion sickness, which commonly occurs during the early days of weightless flight, is presumably attributable to the absence of sustained otolith cues to confirm head movements sensed by the visual system and the semicircular canals.

The process of adaptation to the altered environment, as in adaptation to seasickness, presumably involves some measure of reinterpretation of visual and vestibular signals and prediction of active or passive movement patterns. It is usefully expressed in terms of updating the internal model of the relationship between sensory signals and environmental stimuli. This reinterpretation can be very specific as to the frequency of motion or direction of turning and may provide no protection against sickness or disorientation from other stimuli. Land sickness is represented by unsteady gait, possible disorientation, and slight motion sickness symptoms when walking on steady ground after extensive time at sea. It presumably represents the carry-over of the (now inappropriate) internal model of predicted rolling of the ship and associated visual-vestibular interactions. Although a functioning vestibular system is necessary to produce motion sickness, stimulation of the vestibular appa-

ratus is not required. Motion sickness symptoms may be elicited on the basis of large optokinetic stimuli, such as observed under laboratory conditions (43), in wide-screen movies and in wide-field-of-view fixed-base aircraft simulators (3, 119) or driving simulators. The principal conflict in these situations is between thevection generated by the moving visual field and the lack of confirming semicircular canal or otolith cues. The situation is worsened when the operator is an experienced pilot who presumably anticipates what the appropriate vestibular cues should be in response to a command and reacts to the conflict associated with their nonappearance.

The conflict theory for motion sickness has been extended and cast in control theory terms by Oman (125). Motion sickness symptoms are also generated by head movements under various unusual environments in which the normal pattern of intravestibular or visual-vestibular correlations are disturbed. Conflicting cues are created by wearing left-right reversing prisms or by performance of head movements during weightlessness or in a slowly rotating room. In each of these cases, the vestibular reactions—oculomotor, postural, and perception—do not conform with the observed visual patterns. The subsidence of motion sickness symptoms appears to parallel the development of new internal models and the ability to make appropriate directed movements. The spatial orientation system demonstrates its remarkable versatility in its plastic adaptation to altered environments or even to loss of major portions of the sensory system.

SUMMARY

Spatial orientation is firmly based on the underlying sensory mechanisms and their central integration. For some of the simplest situations, like rotation about a vertical axis in darkness, the dynamic response of the semicircular canals furnishes almost enough information to explain the sensations of turning and stopping. For more complex conditions involving multiple sensory systems and possible conflicts among their messages, a mechanistic response requires significant speculative assumptions. The models that exist for multisensory spatial orientation are still largely of the non-rational parameter variety. They are capable of predicting relationships among input motions and output perceptions of motion, but they involve computational functions that do not now and perhaps never will have their counterpart in central nervous system machinery. The challenge continues to be in the iterative process of testing models by experiment, correcting them where necessary, and testing them again.

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Gravitational Effects on Brain and Behavior

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On earth, the responses of many different sensory organs normally are combined to determine our sensation of which way is down. Visual, vestibular, tactile, proprioceptive, and perhaps auditory cues are combined with knowledge of commanded voluntary movement to produce a single, usually consistent, perception of spatial orientation. Angular stabilization of the eye to reduce retinal image slip and stabilization of head and body position with respect to the vertical to avoid falling are also based upon this multisensory integration process. When tilting one's head to the shoulder, for example, this voluntary movement is confirmed to the brain by signals from the muscle and joint receptors, from the two portions of the balance mechanism of the inner ear: the semicircular canals, which sense angular motion, and the otolith organs, which sense linear acceleration and gravity. In the weightless free-fall condition of orbital space flight the correspondence among the signals is drastically altered. The otolith organs no longer indicate anything meaningful concerning the static orientation of the head. The dense mass of the otoconia no longer pulls the otolithic membrane downhill, bending the hair cell cilia of the maculae when the head is tilted. Rather, like any other linear accelerometer, the output of the otolith organs in weightlessness is limited to indications of the short-duration transient linear accelerations during head movements. Once deprived of the normal static orientation information from the otolith organs, the brain must rely upon other senses to set up a reference frame with respect to which the astronaut can judge his orientation. Visual signals play an increasing role in spatial orientation in weightlessness.

The recent Spacelab flights have provided especially valuable observations on the effects of weightlessness and space flight. During the initial states of adaptation to weightlessness, a conflict exists between the outputs of the otolith organs and the remaining senses, especially associated with voluntary head movements. This conflict is presumed to be the basis of space motion sickness, a malady which affects roughly half of all space travelers and which typically lasts two or three days. As visual cues become dominant, the astronauts begin to orient such that the surface upon which they are working becomes a vertical wall and the place where their feet touch, if they are indeed touching, becomes the floor, or the down reference. Unusual visual orientations, like seeing a fellow crew member upside down, entering a new part of the spacecraft in an unusual orientation, or looking out the window and seeing the earth at the top of the window and the sky at the bottom may prove disturbing and even bring on motion sickness symptoms. Moving visual fields create a greater sense of self motion, and otolith cues begin to be ignored as the astronaut's brain undergoes the reinterpretation of his sensory signals. The limbs no longer have any weight or require any muscle tension when static, other than what is required to overcome internal elasticity. Knowledge of limb position when the muscles are relaxed may be degraded, and the astronaut is occasionally unaware of limb position, which tends to be more flexed than in 1 g. The ability to estimate the mass of objects, in the absence of their weight, is reduced. As measured by the Hoffman reflex during transient accelerations from weightlessness, spinal cord excitability may be greatly reduced. Ocular stabilization during head movements may be impaired, especially for the nodding and tilting motions that normally involve otolith system contributions.

In space, the otolith organs respond only to linear acceleration, as the brain may reinterpret their signals to represent only translation rather than tilt. On earth, such an interpretation would result in the wrong signals to the postural control system to prevent falling, or to reduction in the small compensatory torsional motions of the eye when tilted. Following return to earth astronauts may exhibit wide stance and unsteady gait, and difficulty balancing on a narrow rail with eyes closed. (Reduced blood supply to the brain, associated with cardiovascular deconditioning and blood pooling in the legs when standing just after reentry, may contribute to the unsteadiness.) Head movements may result in unusual illusions of self motion or of ground movement, as the otolith organ signals and perhaps the joint receptors become recalibrated to the terrestrial environment. Return to normal earth functioning may take from one or two days following short flights to several days or weeks for long flights. Part of the recovery is associated with rebuilding the leg muscles which tend to atrophy from disuse in space. Part of the readaptation is also in the brain, which must reinterpret the sensory cues appropriate for earth.

Animal experiments concerned with brain function in weightlessness have been limited. Monkeys can become motion sick, and they may show altered eye movement patterns. Goldfish, when deprived of gravito-inertial orientation forces on the graviceptors, will begin a series of looping swimming motions that may serve to satisfy a drive to remain upright. Spider webs lose their normal regularity.

The sites of adaptation to weightlessness have not yet been determined. Like many other examples of plasticity to sensorimotor rearrangement such as the wearing of reversing prisms, the adaptation is probably central. Other theories involve the end organs themselves. Space experiments can investigate changes in their morphology, such as the number and size of otoconia, or changes in the mechanics of the transducers when the steady load of 1 g is removed. Preliminary results concerning otolith morphology in the rat and otolith organ afferent responses in the frog are inconclusive.

See also Motion Sickness; Vestibular System; Visual-Vestibular Interactions

Further reading

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M.I.T./Canadian vestibular experiments on the Spacelab-1 mission:

1. Sensory adaptation to weightlessness and readaptation to one-g: an overview

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Summary. Experiments on human spatial orientation were conducted on four crewmembers of Space Shuttle Spacelab Mission 1. This introductory paper presents the conceptual background of the project, the relationship among the experiments and their relevance to a "sensory reinterpretation hypothesis". Detailed experiment procedures and results are presented in the accompanying papers in this series. The overall findings are discussed in this article as they pertain to the following aspects of hypothesized sensory reinterpretation in weightlessness: 1) utricular otolith afferent signals are reinterpreted as indicating head translation rather than tilt, 2) sensitivity of reflex responses to footward acceleration is reduced, and 3) increased weighting is given to visual and tactile cues in orientation perception and posture control. Three subjects developed space motion sickness symptoms, which abated after several days. Head movements, as well as visual and tactile cues to orientation influenced symptoms in a manner consistent with the sensory-motor conflict theory of space motion sickness. Six short duration tests of motion sickness susceptibility, conducted pre-flight, failed to predict sickness intensity in weightlessness. An early otolith-spinal reflex, measured by electromyography from the gastrocnemius-soleus muscles during sudden footward acceleration, was inhibited immediately upon entering weightlessness and declined further during the flight, but was unchanged from pre-flight when measured shortly after return to earth. Dynamic visual-vestibular interaction was studied by measuring subjective roll self-motion created by looking into a spinning drum. Results suggest increased weighting of visual cues and reduced weighting of graviceptor signals in weightlessness. Following the 10 day flight, erect posture with eyes closed was disturbed for several days.

Somewhat greater visual field dependence post-flight was observed for two of the crew. Post-flight tests using horizontal linear acceleration revealed an increased variance in detection of acceleration. The ability of the returned crew to use non-visual lateral acceleration cues for a manual control task appeared enhanced over their pre-flight ability for a few days after return.

Key words: Spatial orientation – Vection – Motion sickness – Vestibular – Weightlessness

Introduction

The nearly weightless (*microgravity*) environment of spaceflight provides challenging opportunities for research on sensory-motor adaptation. This paper provides an introduction to the series of interrelated experiments performed on the first Spacelab mission (SL-1) in November 1983 by a team of investigators from MIT and Canada. These investigations, most of which are described in detail in the accompanying five articles, are all aimed at assessing human vestibular and visual responses in space and are intended to clarify the presumed alteration in sensory and motor function in weightlessness. Our working hypothesis, which tied together the various experiments and against which the results are tested, is one of "sensory reinterpretation." A preliminary report was published previously (Young et al. 1984).

Our experiments were designed to help assess human sensory/motor adaptation to weightlessness and readaptation to earth's gravity, and to simultaneously examine the question: is space sickness a motion sickness? The underlying neuroscience research question is how a fully developed sensory

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motor system, which receives redundant information from several sensory mechanisms, reorganizes to account for the environmentally imposed change in the relationship between motor commands and sensory feedback. The results of this research relate to classic studies of sensory rearrangement (e.g. Held and Freedman 1963; Rock 1966; Wallach and Smith 1972; Wallach and Bacon 1976) and to recovery from vestibular lesions (e.g. Igarashi et al. 1970; Fregley and Graybiel 1970). In particular, we ask how pitch and roll perception and postural adjustment are affected by the abnormal pattern of otolith afferent signals which must accompany sustained weightlessness. Our working hypothesis, explained below, was that in the process of sensory adaptation to weightlessness, the low frequency components of the otolith afferent signals (dependent upon head orientation in 1-g) are centrally inhibited or reinterpreted, and that visual and tactile cues consequently play an increasing role in spatial orientation.

Our research also relates directly to the etiology of space sickness, now recognized as a significant problem impacting astronaut performance, safety and well-being. Although space sickness symptoms were not reported in the smaller Mercury and Gemini spacecraft, they have been consistently reported in the Soviet space program (Matsnev et al. 1983) and experienced by Apollo and Skylab crews (Homick and Miller 1975; Graybiel et al. 1977). The incidence among Shuttle crews has exceeded 50% (Homick et al. 1985). It has been parsimonious to assume that the genesis of space sickness is similar to that of motion sickness as experienced on earth (e.g. Benson 1977; Oman 1982b), although conclusive evidence has been lacking and alternative hypotheses have been suggested (see Oman et al., this issue). The etiology of motion sickness is thought to involve the same physiological mechanisms responsible for spatial orientation and body movement control. Based on a sensory-motor conflict theory (Reason 1978; Oman 1982a), motion sickness results when incoming sensory signals no longer match expected patterns learned during previous sensory/motor experience. Because of the environmentally imposed change in graviceptor response to head movements in weightlessness, motion sickness was expected to occur in space. Space sickness would be expected to be exacerbated by real or perceived changes in body orientation, and to subside with a time course paralleling adaptation of sensory-motor systems subserving spatial orientation.

Earlier formal space flight investigations of the influence of weightlessness on human vestibular responses have included the pioneering studies of Graybiel and coworkers (1977) who observed the

absence of motion sickness susceptibility to out of plane head movements made in a rotating chair when tested after the fifth day in space. They also showed the ability to maintain a body oriented reference frame in weightlessness. Homick and Reschke (1977) reported postural instabilities with eyes closed following return of the Skylab astronauts to earth. Other tests of inflight postural stability (Clement et al. 1984) and assessment of the vestibuloocular and optokinetic reflexes have been conducted more recently (Thornton et al. 1985; Watt et al. 1985; Vieville et al. 1986). Relevant Soviet research on man in space has largely been limited, until quite recently, to assessment of motion sickness countermeasures, relationship of spatial illusions to symptoms, and post-flight studies of orientation perception, neuromuscular function and ocular counterrolling (e.g. Yakovleva et al. 1980; Matsnev et al. 1983). Spacelab-1 provided the opportunity for three teams of experimenters (European Space Agency, NASA Johnson Space Center, and MIT/Canada) to perform extensive tests on vestibular function of the same crewmembers during a mission devoted to scientific goals.

A sensory reinterpretation hypothesis for adaptation to weightlessness and readaptation to one-g

A sensory reinterpretation hypothesis formed the basis for our proposed experiments and serves as a useful tool for interpreting the results (Young et al. 1983). It assumes that the functionally appropriate physiological adaptation to weightlessness should involve a reinterpretation of afferent signals originating in the graviceptors, particularly in the otolith organs. These receptors act as linear accelerometers, and respond to the physical input of gravito-inertial force. The adequate input to the otolith organs is the force per unit mass or "specific force" (f), familiar to users of accelerometers for inertial navigation (Fernandez and Macomber 1962). This force, acting on the otolithic membranes, is equal to the vector sum of gravity (g) minus linear acceleration (a). Physically, specific force is the entity tracked by a pendulum. On earth, a non-accelerating body is subject only to the "downward" specific force vector g , and the pendulum points toward the vertical. In orbital flight, a body which is not accelerating relative to the spacecraft experiences a linear acceleration a (as the spacecraft free falls around the earth) equal to the gravitational acceleration g . The specific force acting on the otolith organs is zero, except when head movements are made. Disregarding small gravity gradient effects, a pendulum in earth orbit would assume any arbitrary orientation and velocity previ-

ously imparted to it, and would be of no use in indicating the direction of the center of the earth, or of the spacecraft floor. The otolith organs, of course, continue to provide the central nervous system (CNS) with afferent signals which are modulated by each head acceleration. We believe that on earth the signals from the saccular as well as the utricular otolith organs serve a dual function in spatial orientation and posture control – to estimate the static orientation of the head with respect to the vertical (the traditional graviceptor function) and also to estimate the linear acceleration of the head during movement. The potential ambiguity in interpretation of otolith signals (tilt vs. acceleration) is presumably resolved by CNS integration of information from the semicircular canals, other orientation senses, and knowledge of commanded motion, based on sensory-motor experience in the prevailing environment. In general, the lower frequency components of the otolith signals indicate the direction of the head relative to gravity, whereas the higher frequency components reflect both head tilt and linear acceleration.

In space, where static head orientation doesn't influence otolith organ afferent activity, each head movement produces a specific force stimulus which can swing rapidly in direction even in the absence of any head tilt. The critical question, for which space experiments are necessary, is whether the CNS adapts to accept a radically new relationship between otolith afferent signals and static and dynamic body movement – as appropriate to the new environment. If such adaptation takes place, its time course and its relationship to space motion sickness become important. The removal of a 1 g bias could, in itself, shift the otolith organs to a new portion of their nonlinear operating range, thereby altering their utility in responding to accelerations. One possibility is that the otolith signals are largely inhibited, reducing their influence on posture, eye movements and spatial orientation, and consequently leading to a decrease in the ability to sense linear acceleration of even a transient nature. An alternative hypothesis is that otolith signals are reinterpreted as the CNS learns – via sensory-motor interactions with the weightless environment – that the afferent signals now code only linear acceleration. This hypothesis assumes a robust adaptive capacity and is consistent with much previous research on adaptation to other specific sensory rearrangements (reviewed by Welch 1978). Similar hypotheses have been put forth by other groups (von Baumgarten et al. 1981; Parker et al. 1985). All of our experiments in this program were aimed in one way or another at testing this hypothesis (Oman 1982; Young 1983).

Spacelab-1 mission operations

Spacelab-1 was the first flight of the Spacelab pressurized module, a 30 foot long, manned laboratory for scientific and technical research developed by the European Space Agency (ESA) and carried into orbit in the cargo bay of the Space Shuttle. The "payload crew" of four, which performed all experiments, consisted of two NASA Astronaut Mission Specialists (one of whom had previous Skylab flight experience) and two Payload Specialists chosen by the investigators from the outside scientific community. One of the Payload Specialists was BKL, a vestibular researcher and bioengineer from our MIT laboratory. The Commander and the Pilot did not participate in flight or pre/post-flight experiments. Subjects were male, ranged in age from 35 to 53 years, and were active pilots. They were in good health and were examined and judged normal by our consulting otoneurologist. To preserve anonymity and facilitate data comparison, these subjects are referred to only by letter code A–D throughout this issue. Two crew pairs (A and B, C and D) worked alternating 12 h shifts throughout the mission. Crew circadian rhythms were shifted beginning 14 days before launch, with only partial success. After landing, circadian cycles were abruptly shifted back to local time. It was not possible to control for circadian effects in our testing.

During Spacelab missions, the payload crew lives in the Orbiter and works in the Spacelab, commuting via an access tunnel. The laboratory is maintained at normal sea level atmospheric pressure and air composition, and at comfortable temperature and humidity. Conduct of the scientific mission was substantially different from any flown previously. The investigators on the ground and their astronaut colleagues participated in extensive training, simulation and discussion of scientific goals. They performed as an integrated team, facilitated during the mission for the first time by frequent TV coverage and two-way voice communication. This flexibility permitted numerous repairs and adjustments of experiments (Garriott et al. 1984). Despite the flexibility introduced in Spacelab-1 relative to previous missions, the conduct of experiments was severely restricted in comparison to a normal ground laboratory. The competition for crew time, power, communications and other resources, and the relatively short mission duration prevented substantial extension of measurements.

For this first mission, a wide variety of experiments from the U.S., Canada, eleven European countries and Japan were included (Chappell and Knott 1984). The three closely related sets of vestibular

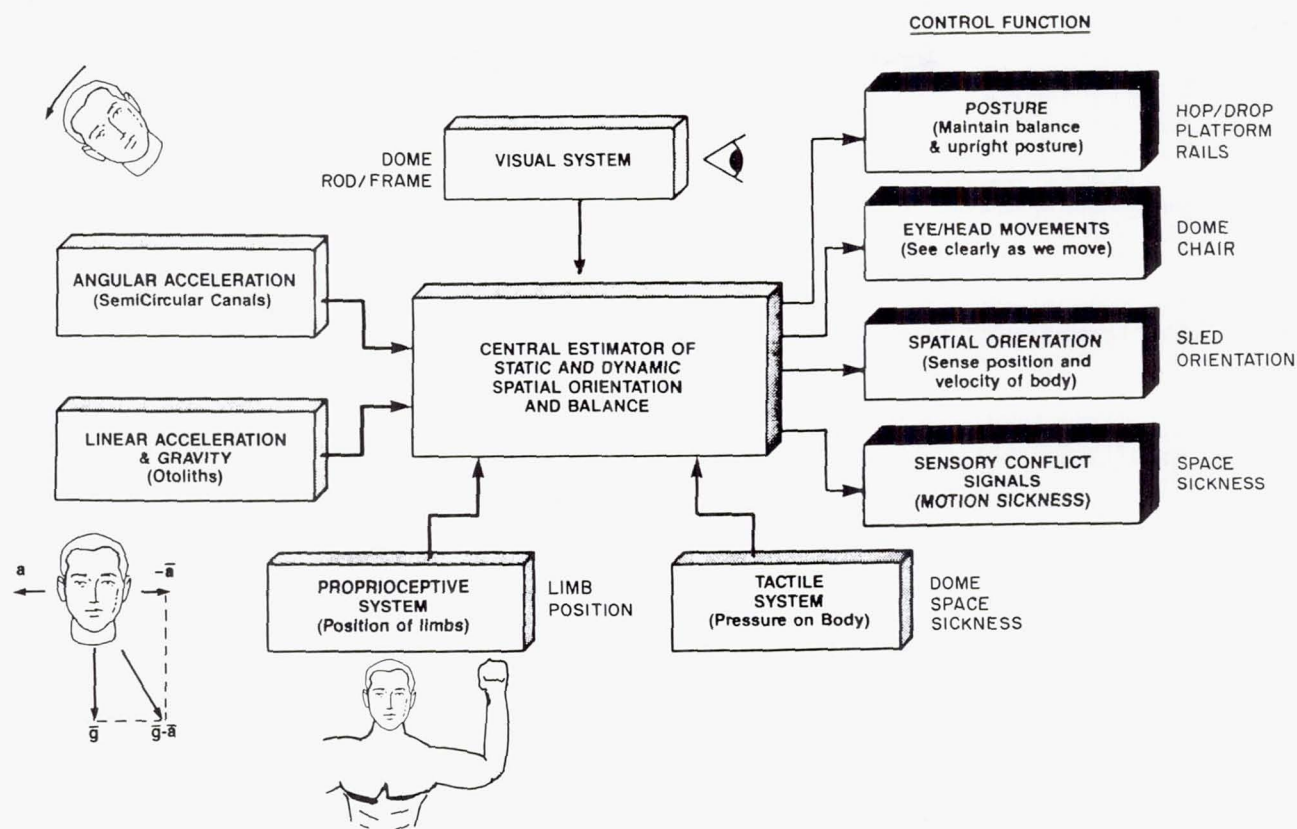


Fig. 1. Scope of the MIT/Canadian Spacelab 1 experiments, by experiment short name, relative to a schematic representation of the role of the vestibular and other senses in control of posture, eye movements and perception of orientation. Experiment short names are keyed to Table 1

lar investigations (von Baumgarten et al. 1984; Reschke et al. 1984; Young et al. 1984) required considerable crew flight time and dominated the pre- and post-flight testing.

Spacelab-1 was launched on November 28, 1983 and was extended from a planned nine days to a mission lasting 10 days, 8 h, 47 min, with a landing at Edwards AFB, California. The landing was delayed by 8 h because of computer malfunctions, severely reducing the crew availability for post-flight testing on the landing day. The NASA nomenclature used for the flight and preserved in the accompanying articles designates the pre-flight days relative to launch. "L minus one" (L-1) is the day before launch. Flight days are numbered beginning with zero. Hence Mission Day 1, or MD1, is the second 24 h in orbit. Post-flight days also are numbered from zero (R+1 is one calendar day after the return day). Mission Elapsed Time (MET) is specified in days/hours : minutes since launch.

Scope and interrelationships of the experiments

The overall scope of our SL-1 experiments and their relationship to the stimuli and outputs of the human system for spatial orientation and balance is indicated in Fig. 1. Individual experiments, investigators and SL-1 performances are shown in Table 1. Each experiment examined a different output to reveal some aspect of the way the CNS adapts to the functional equivalent of removing the gravity vector. The "Rotating Dome" experiment explored central integration of conflicting visual/vestibular/tactile sensory cues by measuring roll self-motion and compensatory eye and head movements stimulated by looking into the open end of a rolling drum. The "Rod and Frame" is a pre-post flight test of static visual field dependence. The "Hop and Drop" experiment studied the otolith-spinal reflex which normally prepares one for a landing from a fall. Electromyographic activity from the gastrocnemius and soleus

Table 1. MIT/Canadian vestibular experiments on SL-1

Experiment	Lead investigator	When performed
1. Visual-vestibular interaction (dome)	Young	Subj. A, B, MD 1, 2, 4, 5, 6, 7; C, D, MD 1 (failed) 3, 6
2. Otolith-spinal reflex (hop/drop)	Watt	Subj. A, MD 0, 1, 6; B, MD 0, 1, 6, 7
3. Awareness of orientation and limb position	Money	*Subj. B, MD 1; C, MD 8
4. Posture control (platform/rails)	Kenyon	Pre-Postflight
5. Motion sickness susceptibility (space sickness)	Oman	Subj. A, B, C, D continuous
6. Perception of linear acceleration (sled)	Arrott	Pre-Postflight (sled scheduled for D-1)
7. Ocular torsion during lateral acceleration	Young	**Subj. C, D, MD 0, MD 7
8. Vestibulo-ocular reflex nystagmus dumping (chair)	Oman	*Subj. A, B, MD 7; C, MD 3, 6

All in-flight tests were also performed pre- and post-flight

MD: Mission Day

* Data still being analyzed – not reported in this issue

** No flight data available due to equipment failure. Full test scheduled for D-1. Pre-postflight data reported with expt. 6

muscles of the leg was measured during footward acceleration provided by stretched elastic cords. The "Position Awareness" experiment measured the influence of weightlessness on both the orientation of perceived objects in the absence of a vertical and the accuracy of proprioceptive cues in determining perceived limb position. The "Space Sickness" investigation clinically characterized space sickness symptoms and studied their relationship to head movements, visual, tactile and proprioceptive cues, and to the shift of body fluids toward the head. A "Posture Platform" and narrow rails were used to measure the post-flight degradation of postural stability. The "Sled" is a linear acceleration device which was used for stimulating eye deviation and ocular torsion, as well as subjective motion during horizontal linear acceleration. A rotating chair was used to stimulate the semicircular canals for study of the horizontal vestibulo-ocular reflex and the "dumping" of post-rotatory nystagmus produced by head pitch.

The experiments conducted on Spacelab 1 were the first of a planned series of related investigations, scheduled for continuation and extension on several additional Spacelab missions in the mid-eighties. For operational reasons the experiments originally planned for use with the Space Sled, a controlled linear acceleration device, were postponed until the D-1 Spacelab mission, accomplished in November, 1985. Related tests were performed on the 1984 Shuttle 41-G Mission (Watt et al. 1985).

Pre-flight testing of the crew for the MIT/Canadian experiments was conducted from 1979 through 1983 at the experimenters' laboratories (MIT, McGill, DCIEM/Toronto) and at NASA's Johnson Space Center, Kennedy Space Center, and Dryden Flight Research Facility (DFRF). Of particular value for protocol development, training and baseline data

collection were the series of four sets of parabolic flight tests producing repeated 20–25 s periods of weightlessness in NASA's KC-135 aircraft. Pre-flight and post-flight testing by all life science experimenters was conducted at an especially constructed Baseline Data Collection Facility at DFRF at approximately 152, 122, 65, 44 and 10 days before launch. Subjects A and B were tested within hours of landing, and all four subjects were tested on 1, 2, 4 and 6 days after return. Parabolic flights to assess 0 g motion sickness susceptibility and reorientation illusions were performed pre-flight, and 3 days, and 1 year after landing.

Results and discussion

The results of our experiments on Spacelab 1, discussed in detail in the accompanying papers, must be interpreted cautiously because the experiments were conducted on only 2–4 subjects, and with fewer repetitions and frequently under less well controlled conditions than desired. These results, when taken together with findings from other related experiments, appear generally consistent with the sensory reinterpretation notion. We are aware of no evidence pointing to pathological alteration of sensory function at the end organ.

Early in the SL-1 flight 3 of 4 subjects developed space sickness symptoms, which largely resembled those of prolonged motion sickness, superimposed on the effects of fluid shift towards the head. Symptoms abated after 2–3 days. Short duration pre-flight motion sickness susceptibility tests did not predict in-flight sickness intensity. However, head movements, especially in pitch, as well as visual and tactile cues to orientation, influenced symptom level

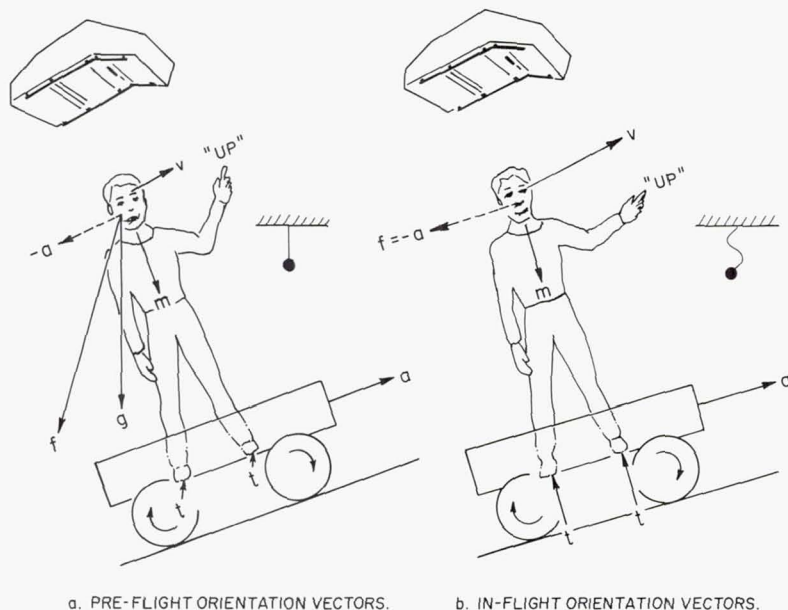


Fig. 2a, b. Schematic representation of the sensory vectors which are used in determining human spatial orientation. In **a**, the subjective zenith is arrived at by a vector sum of the various sensory contributions, but is dominated by the gravito-inertial vector (f). If the subject, shown standing on a moving wagon, were not accelerating, this would indeed be vertical (g). The subjective vertical is also biased slightly by the influence of vertical or horizontal elements in the visual field (v), by localized tactile cues (t), and by one's own body axis (m). The strength of these other cues depends on the individual. In **b**, which represents the similar situation in weightlessness, the crucial difference is that the gravito-inertial vector now represents only linear acceleration (a). The subjective zenith, or local reference axis if "up" has lost all meaning, now ignores the gravito-inertial vector in favor of the stronger visual, tactile and body centered axes. Tactile cues normal to support surfaces, such as illustrated in **b**, could be developed by a loading mechanism such as stretched elastic cords (not shown) or briefly by extension of the legs. Differences among the individual crew members in the relative strength of these vectors is reflected in the range of orientation styles

in ways consistent with the sensory conflict theory for motion sickness and with the hypothesis of sensory reinterpretation.

Changes in sensory-motor function were observed both during the flight and extensively following the landing. Otolith-spinal reflex responses to footward acceleration with head erect were inhibited when tested early in the flight, and declined further during the week in weightlessness. However, in the tests performed several hours after landing the otolith-spinal reflex had returned to pre-flight levels. Similarly, the short latency reflex reactions to destabilization of standing on the posture platform were unchanged post-flight, although the longer latency responses demonstrated postural instability, with eyes closed, on both the platform and on the rails tests. The Rotating Dome experiment data suggest increased weighting of visual cues and tactile cues, and reduced influence of graviceptor signals in determination of orientation in weightlessness. Post-flight measurements also suggested a slight increase in static visual field dependence. Proprioception may have been degraded in flight. Post-flight reaction to horizontal linear acceleration revealed a reduction in dynamic ocular counterrolling, and increased variability in the detection of low level accelerations, but an enhanced ability to use suprathreshold acceleration cues to null lateral position in a closed loop, non-visual, tracking task.

As illustrated in Fig. 2, the human estimation of body position and postural reactions is thought to change in weightlessness to make use of the varied sensory inputs in a manner which is fundamentally appropriate to the microgravity condition. In particular, it appears likely that at least three separate aspects of such reinterpretation may be present: tilt acceleration reinterpretation, reduced postural response to z-axis linear acceleration, and increased attention to visual cues. In the course of the reinterpretation, motion sickness symptoms, caused by the original sensory motor conflicts, gradually disappear.

As illustrated in Fig. 2a, for pre-flight spatial orientation, the subject relies heavily on the static gravito-inertial vector for his perception of the vertical, which can be displaced by a low frequency acceleration (e.g. Mach 1875; Howard and Templeton 1966; Schöne 1980; Young 1984). However, each individual has his perception of the upright influenced, to varying degrees, by the presence of elements in the visual field, especially those normally associated with the vertical (e.g. Witkin 1958; Howard 1982) and by localized tactile cues such as pressure on the soles of the feet. Moving visual scenes (not shown in the figure) can also create a sense of body self-motion. Furthermore, each individual has a tendency to align the perceived vertical toward the head or feet along the torso long axis.

This tendency is represented by an idiotropic body axis vector and is assumed to vary in strength among individuals (Mittelstaedt 1983).

These sensory vectors must be reinterpreted for spatial orientation in weightlessness. As shown in Fig. 2b, the gravito-inertial vector now is merely the opposite of linear acceleration relative to the spacecraft. If it were to continue to dominate the perception of tilt orientation, the astronauts would experience 180 degrees of roll or pitch each time they accelerated and decelerated while translating through the spacecraft, which was never reported. Instead, we believe that the signals from the graviceptors are reinterpreted to represent linear translation, as required for locomotion accuracy in space, and as carried over to the post flight closed loop acceleration nulling tests. In-flight postural reaction to changes in acceleration, at least along the body z-axis (Watt et al., this issue; Reschke et al., this issue) show a decrease in sensitivity, which is consistent with the absence of a need to prepare the "anti-gravity muscles" for a fall. (It remains to be determined whether this inhibition is limited to z-axis acceleration.) Upon return to earth this reinterpretation of graviceptor cues leads to a decreased ability to stand up with eyes closed, except within a very narrow cone of static stability near the upright. Actual head tilt may be perceived as a lesser tilt post-flight, combined with linear acceleration in the opposite direction, leading to destabilizing postural reactions in the wrong direction. Post-flight changes in postural control strategy may be related to this tilt/translation reinterpretation (Kenyon and Young, this issue, Reschke et al. 1984). Ocular counterrolling, which is a normal compensatory response to a tilted gravito-inertial vector, is also shown to be reduced post-flight dynamically (Arrott and Young, this issue) and statically (von Baumgarten et al. 1984; Parker et al. 1985; but not Yakovleva et al. 1980). Post-flight perceived tilt, in the dark, is reduced (Benson et al. 1984) as predicted by the hypothesized carry-over of the otolith reinterpretation, and dynamic tilt was reported on other crews to lead to a strong translation sensation (Parker et al. 1985, who independently arrived at a similar otolith tilt/translation reinterpretation hypothesis).

In the absence of usable graviceptor information regarding body orientation in weightlessness, the nervous system must pay increased attention to the remaining sensory orientation signals. Subjective reports from crew members indicate large variations in individual styles, but never a prolonged sense of absence of a reference frame or "disorientation". The increased length of the "visual" vector in Fig. 2b is intended to represent the increased weighting

given to dynamic visual inputs to self motion (the dome experiment) and to static elements such as the floor or ceiling, another crew member, or the earth (Oman et al., this issue). In many cases the relative weighting may be a complete domination by the visual, body control or tactile vector in weightlessness. Large individual differences in visual field influence in weightlessness are reflected in the post-flight increases in field dependence. Similarly, localized tactile cues, such as pressure on the feet in the Dome and the Hop/Drop experiments or on the buttocks and back when wedging into a corner, serve to take on an increasing role in determining spatial orientation and a sense of well-being. Finally, the influence of the postulated body-axis orientation vector, which could allow some crew members to orient their reference frame to their body long axis in weightlessness, is greater than pre-flight because of the reinterpretation of the graviceptor cues.

Acknowledgements. The successful conduct of a manned space experiment requires contributions of many more people than a comparable ground experiment. Among those who made extraordinary contributions to the entire project were R. Clark, J. Rummel, M. Buderer, J. Homick, E. Peck, M. McEwen, J. Evans, D. Griggs, F. Amlee, W. Beaver, and the Science Monitoring Staff of NASA's Johnson Space Center, G. Salinas and B. Walters of G.E. Matsco, H. Craft, Mission Manager, C. Lewis, W. Bock, and the operations cadre from NASA's Marshall Space Center, G. Soffen, A. Nicogossian, T. Perry and L. Chambers of NASA Headquarters, the support staff at NASA's Kennedy Space Center and Dryden Flight Research Facility, the flight crew and the alternate payload specialists, W. Ockels and M. Lampton, the MIT Center for Space Research Laboratory for Space Experiments - E. Boughan and W. Mayer, project managers, the students of the Man-Vehicle Laboratory, MVL Staff, R. Renshaw and S. Modestino, secretaries K. Campbell, M. Armour and M. Williams and MIT administrators, W. Rosenblith and J. Kerrebrock. General guidance and support was supplied by our Scientific Advisors: A. Graybiel, F. Guedry, W. Johnson, and G. Melvill Jones. The overall program was supported by NASA contract NAS9-15343 to MIT and by DCIEM and the MRC of Canada. Individual acknowledgements for each experiment follow those papers.

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SECTION II - ABSTRACTS

This section provides a reference source for additional information about the research that has been conducted by the SLS-1 investigator teams. Because all the investigators have published widely, space does not permit full presentation of all their published work. Instead, in addition to the selected papers presented in Section I, abstracts and bibliographic citations of additional papers by the investigators are presented in this section. These papers often contain more detailed information, or concentrate on more specific topics, than the overview papers in Section I.

Gmünder FK, and Cogoli A. Cultivation of single cells in space. *Appl Micrograv Tech I* 1988;3:115-122. The purpose of this review is to present an updated and comprehensive analysis of the experiments with single cells performed in space. Especially the results of the investigations performed in Biorack on the D-1 mission clearly show that important cellular functions are changing in microgravity. Cell proliferation, differentiation, metabolism, membrane properties, and cytoplasmic streaming underwent significant alteration during exposure to space flight conditions in a variety of single cells cultures spanning from bacteria to mammalian cells. These findings open new and interesting perspectives to basic and applied research in microgravity. The focus of this paper is on the cultivation of mammalian cells in space laboratories and on the related instrumentation. While Biorack is a useful and efficient instrument for simple studies in Spacelab, the development of new facilities like incubators with automated fixation devices as well as of more complex bioreactors is strongly recommended.

Sieber-Blum M, Kumar SR, and Riley DA. In vitro differentiation of quail neural crest cells into sensory-like neuroblasts. *Dev Brain Res* 1988;39:69-83. This study shows that quail neural crest cells can differentiate in vitro into sensory-like neuroblasts. The putative sensory neuroblasts were large and spherical, possessing large diameter, bipolar or pseudo-unipolar, long processes that lacked multiple varicosities characteristic of autonomic neurons. They bound HNK-1, a monoclonal antibody against a cell surface epitope expressed by early neural crest cells but not by young neural tube-derived cells. Many of the sensory-like neuroblasts had substance P (SP)-like immunoreactivity. Some exhibited histochemical carbonic anhydrase activity; carbonic anhydrase is shown in this study to stain a subpopulation of spinal sensory neurons in adult quail and embryos 9 days and older, whereas ventral root axons and neurons in sympathetic ganglia are non-reactive at all ages. Double staining indicated that unlike the multipolar neuroblasts developing in the same cultures, SP-like immunoreactive neuroblasts do not contain detectable levels of tyrosine hydroxylase or dopamine- β -hydroxylase. Finally, the neuronal nature of the cultured sensory-like neuroblasts was further documented by double labeling for antibodies against the 68 kDa neurofilament polypeptide and substance P.

CELL BIOLOGY

NEUROVESTIBULAR

Arrott AP, and Young LR. M.I.T./Canadian vestibular experiments on the Spacelab-1 mission: 6. Vestibular reactions to lateral acceleration following ten days of weightlessness. *Exp Brain Res* 1986;64:347-357. Tests of otolith function were performed pre-flight and post-flight on the science crew of the first Spacelab Mission with a rail-mounted linear acceleration sled. Four tests were performed using horizontal lateral (y-axis) acceleration: perception of linear motion, a closed loop nulling task, dynamic ocular torsion, and lateral eye deviations. The motion perception test measured the time to detect the onset and direction of near threshold accelerations. Post-flight measures of threshold and velocity constant obtained during the days immediately following the mission showed no consistent pattern of change among the four crewmen compared to their pre-flight baseline other than an increased variability of response. In the closed loop nulling task, crewmen controlled the motion of the sled and attempted to null a computer-generated random disturbance motion. When performed in the light, no difference in ability was noted between pre-flight and post-flight. In the dark, however, two of the four crewmen exhibited somewhat enhanced performance post-flight. Dynamic ocular torsion was measured in response to sinusoidal lateral acceleration which produces a gravito-inertial stimulus equivalent to lateral head tilt without rotational movement of the head. Results available for two crewmen suggest a decreased amplitude of sinusoidal ocular torsion when measured on the day of landing (R+0) and an increasing amplitude when measured during the week following the mission.

Kenyon RV, and Young LR. M.I.T./Canadian vestibular experiments on the Spacelab-1 mission: 5. Postural responses following exposure to weightlessness. *Exp Brain Res* 1986;64:335-346. The four science crewmembers of Spacelab-1 were tested for postural control before and after a 10-day mission in weightlessness. Previous reports have shown changes in astronaut postural behavior following a return to earth's 1-g field. This study was designed to identify changes in EMG latency and amplitudes that might explain the instabilities observed post-flight. Erect posture was tested by having the subject stand on a pneumatically driven posture platform which pitched rapidly and unexpectedly about the ankles causing dorsi- and plantarflexion. Electromyographic (EMG) activity from the tibialis anterior and the gastrocnemius-soleus muscles was measured during eyes open and eyes closed trials. The early (pre 500 ms) EMG response characteristics (latency, amplitude) in response to a disturbance in the posture of the subject were apparently unchanged by the 10 days of weightlessness. However, the late (post 500 ms) response showed higher amplitudes than was found pre-flight. General postural control was quantitatively measured pre- and post-flight by a "sharpened Romberg Rails test". This test showed decrements in standing stability with eyes closed for several days post-flight.

Money KE, Watt DG, and Oman CM. Preflight-and postflight motion sickness testing of the Spacelab 1 crew. In: MOTION SICKNESS: MECHANISMS, PREDICTION, PREVENTION AND TREATMENT. AGARD CP-372 1984;33-1--33-8. The four Spacelab 1 payload crew members, as experimental subjects, were exposed to a variety of motion sickness tests. Contrary to expectation, the crew member who was most susceptible to these tests was the least susceptible to space motion sickness, and the crew member who was most susceptible to space motion sickness was one of the least susceptible to these tests. On the third day after returning from the mission, one of the preflight tests (KC 135) was repeated, and all of the crew members were found to be non-susceptible. Statements of generalities will have to wait for the accumulation of more experimental subjects.

Oman CM. Spacelab experiments on space motion sickness. *Acta Astronautica* 1987;15(1):55-66. Recent research results from ground and flight experiments on motion sickness and space sickness conducted by the Man Vehicle Laboratory are reviewed. New tools developed include a mathematical model for motion sickness, a method for quantitative measurement of skin pallor and blush in ambulatory subjects, and a magnitude estimating technique for ratio scaling of nausea or discomfort. These have been used to experimentally study the time course of skin pallor and subjective symptoms in laboratory motion sickness. In prolonged sickness, subjects become hypersensitive to nauseogenic stimuli. Results of a Spacelab-1 flight experiment are described in which four observers documented the stimulus factors for and the symptoms signs of space sickness. The clinical character of space sickness differs somewhat from acute laboratory motion sickness. However SL-1 findings support the view that space sickness is fundamentally a motion sickness. Symptoms were subjectively alleviated by head movement restriction, maintenance of a familiar orientation with respect to the visual environment, and wedging between or strapping onto surfaces which provided broad contact cues confirming the absence of body motion.

Oman CM, Lichtenberg BK, Money KE, and McCoy RK. M.I.T./Canadian vestibular experiments on the Spacelab-1 mission: 4. Space motion sickness: symptoms, stimuli, and predictability. *Exp Brain Res* 1986;64:316-334. Space sickness symptoms were observed by 4 specially trained observers on Spacelab-1. Three reported persistent symptoms, and vomited repeatedly during the first and/or second day of flight. Head movements on all axes were provocative, particularly in pitch and roll. Head acceleration data recorded from 2 symptomatic crewmen showed that after several hours of physical activity in orbit, symptoms appeared and thereafter both crewmen were compelled to limit head movements. Firm body contact with motionless surfaces helped alleviate symptoms. When crewmembers floated into unfamiliar body orientations in the cabin, inherent ambiguities in static visual orientation cues sometimes produced spatial reorientation episodes which were also provocative. Symptoms largely resembled those of other forms of prolonged motion sickness, superimposed upon other symptoms attributable to fluid shift. All 4 eventually used anti-motion sickness drugs. When they did, vomiting frequently was reduced. By the 4th day, symptoms subsided, and head accelerations again increased in magnitude and variability. Sickness intensity in orbit was not predicted by statistically concordant results of 6 acute preflight susceptibility tests. However, results from a longer duration preflight prism goggles test showed an apparent correlation. All subjects were asymptomatic making head movements in parabolic flight 4 days after the mission, but not 1 year later. Overall, results support the view that space sickness is a motion sickness.

Oman CM, Young LR, Watt DGD, Money KE, Lichtenberg BK, Kenyon RV, and Arrott AP. MIT/Canadian Spacelab experiments on vestibular adaptation and space motion sickness. In: BASIC AND APPLIED ASPECTS OF VESTIBULAR FUNCTION (Eds.) Hwang JC, Daunton NG, and Wilson VJ. Hong Kong: Hong Kong University Press. 1988;183-192. Experiments on sensory-motor adaptation to weightlessness and re-adaptation to 1 g were conducted on Space Shuttle/Spacelab Missions 1 and D-1 by a team of investigators from MIT and Canada. Results from both missions are reviewed in the context of a sensory re-interpretation hypothesis and the conflict theory for motion sickness.

Ross MD. Anatomic evidence for peripheral neural processing in mammalian graviceptors. *Aviat Space Environ Med* 1985;56(4):338-343. Ultrastructural study of utricular and saccular maculas demonstrates that their innervation patterns are complex. There is a clustering of type I and type II hair cells based upon a sharing of afferents, a system of efferent-type beaded fibers that is of intramacular (mostly calyceal) origin, and a plexus-like arrangement of afferents and efferents at many sites in the neuroepithelium. Results

suggest that information concerning linear acceleration is processed peripherally, beginning at the hair cell level, before being sent to the central nervous system. The findings may supply a structural basis for peripheral adaptation to a constant stimulus, and for lateral inhibition to improve signal relative to noise.

Ross MD. Implications of otoconial changes in microgravity. *Physiologist* 1987;30(1,Suppl):S90-S93. Otoconia of maculas of Sprague-Dawley rats (Taconic Farms) flown aboard Spacelab-3 showed no signs of demineralization. Other findings were accumulations of miniature otoconia at the lateral border of utricular patches and a smoothing of surfaces of saccular otoconia. These features were not observed in age- and weight- matched ground controls. Subsequent study showed otoconial asymmetry to be normal in this strain. Further research in space, taking this into account, is clearly required. Findings of ground-based studies would suggest that neural structures of maculas are adaptable to microgravity but might show changes over time. Moreover, maculas have the potential for integration of the sort ascribed to brain and retina, although on a less complex scale. They may act as comparators, and asymmetry may be an important property. Coordinated studies in space and on the ground could lead to new understanding of how maculas function and adapt to new acceleratory environments; and to insights about the functioning of neural tissue in general.

Ross MD. Mammalian macular organization: a model information network. *Adv Oto Rhino Laryngol* 1988;41:142-145. Recent ultrastructural findings in rat maculas have demonstrated that type II hair cells are integrated into the neural circuitry innervating type I cells. This work also showed that three kinds of afferent terminal patterns occur. These are U-, M/U- and M-types, illustrated diagrammatically in figure 1. These and other observations were interpreted to indicate that complex processing of information takes place in the mammalian macula. More recent study of a long series of sections through rat utricular macula supports the notion that type II hair cells link calyces and distribute information. This means that maculas are neural networks morphologically organized for parallel processing of linear acceleratory signals. This paper focuses on macular neural connectivity, especially on networks in pars externa encompassing the three different kinds of nerve/terminal patterns. The evident weighting in directional flow of information and possible physiological implications of the findings are discussed.

Ross MD. Morphological evidence for parallel processing of information in rat macula. *Acta Otolaryngol* 1988;106:213-218. Study of montages, tracings and reconstructions prepared from a series of 570 consecutive ultrathin sections show that rat maculas are morphologically organized for parallel processing of linear acceleratory information. Type II cells of one terminal field distribute information to neighboring terminals as well. The findings are examined in light of physiological data which indicate that macular receptor fields have a preferred directional vector, and are interpreted by analogy to a computer technology known as an information network.

Ross MD. Striated organelles in hair cells of rat inner ear maculas: description and implication for transduction. *Physiologist* 1982;25(6,Suppl):S113-S114. Use of unusual fixation procedures resulted in display of the several striated organelles that are present in hair cells of the inner ear. In the vestibular system these include the striated rootlets of the kinocilia, the striated cuticular plate and its attachments to cell membrane at the zonula adherens, and a striated neck organelle (SNAP) that is present only in Type I hair cells. The possible roles of these organelles in vestibular hair cell transduction are considered here. It is suggested that the kinociliary apparatus serves as a pacemaker for hair cell activity, and that the cuticular

plate functions in part to coordinate kinociliary and stereociliary interactions. The plate also could transmit signals for the apical to the lateral cell membrane and, if contractile, could amplify small signals and produce graded hair cell responses. SNAP is situated at the plasma membrane under the upper end of the calyx nerve ending where it could modulate hair cell electric conductance.

Ross MD, and Bourne C. Interrelated striated elements in vestibular hair cells of the rat. *Science* 1983;220:622-624. Unusual fixation procedures revealed a series of interrelated striated organelles in type I and type II vestibular hair cells of the rat; these organelles seemed to be less well developed in cochlear hair cells. The findings suggest that contractile elements may play a role in sensory transduction in the inner ear, particularly in the vestibular system.

Ross MD, Donovan KM, and Chee O. Otoconial morphology in space-flown rats. *Physiologist* 1985;28(6,Suppl):S219-S220. One question to be answered by spaceflight is whether gravity receptors will show degenerative changes during short- or long- term exposures to microgravity. Weightlessness imposes a new bias against which translational accelerations must be judged. Will the system adjust to this change in bias by undergoing visible alteration or degeneration, or will adaptive processes be more subtle? This ultrastructural study of inner ears obtained from space-flown rats and from age-matched, ground-based controls is our first step in attempting to answer the questions posed. The rats were used primarily for testing the animal holding facility under space-flight conditions rather than for elucidating vestibular responses to microgravity. Nevertheless, it can be stated conclusively that short-term exposure to weightlessness (7 days) does not result in degeneration of macular receptors. It is possible, instead, that a slight increase in otoconial mass occurred. In the utricle, this appeared to be through otoconial neogenesis and in the saccule, by growth of existing otoconia.

Ross MD, Donovan KM, and Rogers C. Peripheral sensory processing in mammalian gravity receptors: observations of ciliary tuft configurations. In: THE VESTIBULAR SYSTEM: NEUROPHYSIOLOGIC AND CLINICAL RESEARCH (Eds.) Graham MD, and Kemink JL. New York: Raven Press. 1987;119-124. Recent findings from our laboratory have provided a morphological basis for peripheral processing of linear acceleratory information by vestibular gravity receptors. Type I and type II cells were organized into functional groups through shared innervations in both the anterior part of the utricular and the inferior portion of the saccular maculae. We now have completed a study of 148 serial sections through the anterior part of the saccular macula. Here, as in the other areas studied, innervation of the type II cells was dependent on the neural circuitry supplying type I cells. During the course of this work, our attention was drawn to the orientations, sizes, and configurations of the ciliary tufts of hair cells comprising functional groupings. Type II hair cell ciliary tufts always consisted of finer and shorter stereocilia than those present on type I cells, and hair cells comprising clusters did not appear to be dynamically polarized in parallel. Ciliary orientations and differences in height have physiological implications for neural processing equal in importance to the neural circuitry underlying type I and type II cell integration. We have therefore begun a coordinated investigation using both transmission electron microscopy (TEM) and scanning electron microscopy (SEM) to determine the dynamic polarizations of clustered cells and to shed some light on the possible meaning of two kinds of hair cells integrated into the same neural circuitry. This paper focuses on preliminary findings of our SEM study.

Ross MD, and Peacor DR. The nature and crystal growth of otoconia in the rat. *Ann Otol Rhinol Laryngol* 1975;84(1):22-36. Several types of otoconia are present in the macular regions of young rats. These include multifaceted, transitional and rounded body forms, some variant otoconia and a few rhombohedrons. The adult form has typically rounded but nonsmooth body surfaces and pointed ends with three planar faces. The multifaceted and transitional otoconia fracture and etch more readily than do the adult type. The differences in properties of the otoconia are considered in the light of known facts concerning inorganic crystal nucleation and growth. This integrated approach indicates that many otoconia originate by seeding of multiple subunits on an organic substrate and develop by the mechanism of parallel growth. The basic structural unit is the rhombohedron. By analogy to inorganic crystals of calcite, it would seem that the typical otoconium grows on the end faces but growth on the side faces is suppressed by some unknown chemical factor. Some otoconia are exceptions, evidently seeding and growing in the pure rhombohedral form. Decalcification of cleaved otoconia shows that organic material is incorporated during growth. The observations are interpreted to indicate that organic substance influences growth and achievement of the adult otoconial form.

Ross MD, Peacor DR, Johnsson LG, and Allard LF. Observations on normal and degenerating human otoconia. *Ann Otol Rhinol Laryngol* 1976;85(3):310-326. Specimens of human otoconia obtained from autopsy material and representing various stages from fetal to advanced old age, were studied by microdissection, scanning electron microscopy, electron microprobe analysis, and x-ray powder diffraction. The typical adult otoconial configuration is a cylindrical, finely serrated body with pointed ends; crystallographically, it corresponds to a single crystal of calcite. Other, less numerous types include joined otoconia, pure rhombohedrons and multifaceted, presumably immature forms. Many otoconia achieve the adult configuration during fetal development. The multifaceted otoconia are most numerous, and the rhombohedrons proliferate, during childhood in the utricle. Crystals from both end organs are virtually identical in composition in the young adult, but saccular otoconia are the larger. In middle and advanced age the otoconia decrease in number, especially in the saccule. Saccular otoconia degenerate progressively in a posteroanterior direction across the macula; they assume a specific, fibrous, hollowed-out appearance, which is not duplicated by either chemical etching or autolysis. Neogenesis and growth of otoconia appear to occur postnatally, with different characteristic growth potentials for those of the saccule and the utricle. Age-related saccular otoconial degeneration appears to involve the organic material, which disappears either before or simultaneously with the mineral substance.

Ross MD, and Pote KG. Some properties of otoconia. *Phil Trans R Soc Lond* 1984;B304:445-452. Otoconia are dynamic mineral deposits present in the gravity receptors of most vertebrates; fishes often have a single large mass called an otolith instead. Otoconia generally have the appearance of single crystals but contain organic and inorganic components, the mineral being almost exclusively a polymorph of calcium carbonate. The two phases are closely interrelated structurally. Ultra-high resolution transmission electron microscopy of rat otoconia showed them to be mosaic biominerals. The crystallites were 50-100 nm in diameter, had some rounded edges, and were highly ordered into laminae. This suggests that crystallite seeding and growth is organic matrix mediated. Crystallite asymmetry may also indicate piezoelectricity. A further finding of similarities in electron beam diffraction patterns obtained from some frog and rat otoconia could mean that the calcite of mammalian units mimics aragonite. A comparative study showed that turtles, which are close to the stem line for mammals, had calcite-type otoconia in the utricle. Alligators, which share a common ancestry with birds, had this type otoconium in all three gravity receptors, although saccular otoconia had a variety of forms. The nature of the mineral is unknown. The biochemical composition of the organic otoconial material is under study, to learn how mineral deposition is regulated.

Proteins of rat otoconial complexes ranged between *ca.* 16500 and over 100 000 Da in molecular mass and were similar in saccular and utricular otoconial complexes. Our new analysis of the amino acid composition of the complexes by high performance liquid chromatography showed the complexes to be high in the acidic and low in the basic amino acids. This is comparable to what has already been reported for other biomineralized materials that contain calcite.

Ross MD, Pote KG, Cloke PL, and Corson C. *In vitro* $^{45}\text{Ca}^{++}$ uptake and exchange by otoconial complexes in high and low K^+/Na^+ fluids. *Physiologist* 1980;23(6,Suppl):S129-S130. Recently, data have been accumulating to indicate that saccular and utricular otoconial complexes of the gravity receptor organs are dynamic and interact constantly with their environment. This study investigates the possibility that the ionic composition of the surrounding fluid influences calcium ion binding and release, and explores the importance of the K^+/Na^+ ratio. Two *in vitro* methods were developed, the first of which employed artificial endolymph and perilymph while ionically balanced fluids in which only the K^+/Na^+ was altered were used in the second. The ability of rat complexes to take up $^{45}\text{Ca}^{++}$ during incubation with these fluids was assessed using liquid scintillation spectrometry. *In vitro* uptake of $^{45}\text{Ca}^{++}$ was greater in fluids with a high K^+/Na^+ ratio than in fluids in which the ratio was low. The ability of the complexes to take up $^{45}\text{Ca}^{++}$ appeared to decline with age.

Ross MD, Pote KG, Rarey KE, and Verma LM. Microdisc gel electrophoresis in sodium dodecyl sulfate of organic material from rat otoconial complexes. *Ann NY Acad Sci* 1981;374:808-819.

Ross MD, Rogers CM, and Donovan KM. Innervation patterns in rat saccular macula. *Acta Otolaryngol* 1986;102:75-86. Serial sections through the anterior part of rat saccular macula were reconstructed as montages. Findings are that type II hair cells are integrated into the neural circuitry of type I cells, chiefly by synapses with neighboring calyces and their collaterals; and that complex interactions between afferent- and efferent-type nerve elements take place. Three basic types of nerve/calyx pattern are present: U-type nerves lose their myelin before they enter the macula and have complex calyces with several collaterals; M-type nerves are myelinated up to the calyx, which lacks collaterals; and M/U-type nerves have short, unmyelinated segments proximal to their calyces, which have few collaterals. Both afferent- and efferent-type collaterals spring from calyces, chiefly for those of U-type nerves. Type II cells are presynaptic both to electron-lucent and to vesiculated terminals; some synapses are reciprocal. Electronlucent boutons sometimes are presynaptic to calyces and to type II hair cells; and morphologically afferent-to-afferent kinds of synapses occur in the neuroepithelium. The anatomical findings indicate that complex information processing must occur in mammalian gravity receptors.

Ross MD, and Williams TJ. Otoconial complexes as ion reservoirs in endolymph. *Physiologist* 1979;22(6,Suppl):S63-S64. Otoconia of the gravity receptors and their membranes are complex mineral deposits of calcite crystals and organic substance. $^{45}\text{Ca}^{2+}$ uptake and exchange in these complexes and in bone mineral were studied in young adult Wistar rats using microdissection procedures, pooled otoconial samples and the sensitive method of liquid scintillation spectrometry. Intraperitoneal injection of $^{45}\text{Ca}^{2+}$ (4 mCi/kgm body weight) resulted in rapid uptake into saccular complexes (15 mins) but uptake into utricular complexes took longer (up to two hrs). Although retention of $^{45}\text{Ca}^{2+}$ in saccular complexes was higher throughout the one month experimental period, major uptake had occurred in both complexes by 4 hrs. This time frame is constant with that for entry of the major part of an injected dose of $^{45}\text{Ca}^{2+}$ into bone. $^{45}\text{Ca}^{2+}$ declined in otoconial

complexes after 4 days but remained high in bone mineral. The results indicate that otoconial complexes are dynamic mineral deposits but function in distinct environments at the two sites. It is suggested here that otoconial complexes not only provide mass to enhance gravity receptor function but also act as ion reservoirs, contributing to the ionic stability of the endolymph.

Salamat MS, Ross MD, and Peacor DR. Otoconial formation in the fetal rat. *Ann Otol Rhinol Laryngol* 1980;89(3):229-238. The development of otoconia in the fetal rat was investigated by scanning and transmission electron microscopy and by x-ray elemental analysis. The transmission electron microscopical results indicate that primitive otoconia are highly organic appearing but are trigonal in cross section, indicating that they already possess a three-fold axis of symmetry and a complement of calcite. These otoconia develop into spindle-shaped units which accrue fibrous, organic material at an angle to their surfaces. Dumbbell-shaped otoconia, with distinct central cores and peripheral zones, result. These otoconia then mature to the adult crystal configuration having a more cylindrical body and pointed ends. The existence of trigonal, spindle- and dumbbell-shaped otoconia was verified by scanning electron microscopy of fresh-frozen material. Tissues prepared for transmission electron microscopy proved (by elemental analysis) to have been decalcified inadvertently, fortuitously revealing the arrangement of the organic material. Subsequent transmission electron microscopy of dumbbell-shaped otoconia not exposed to fluids during embedment showed that calcite deposits mimicked the arrangement of the organic material. X-ray elemental analysis demonstrated that calcium was present in lower quantities in the central core than peripherally. Findings are interpreted to indicate that organic material is essential to otoconial seeding and directs otoconial growth.

Shelhamer M, Marino LA, Young LR, Arrott AP, and Wiseman JJ. Normative study of Spacelab preflight/postflight vestibular test battery. *Aviat Space Environ Med* 1987;58(9,Suppl):A236-A239. A study was designed to establish baseline normative responses to the MIT/Canadian Spacelab vestibular test battery. Three tests used a linear acceleration sled to measure otolith function: 1) perception of linear motion (threshold determination); 2) compensatory eye movements (linear VOR); 3) closed-loop nulling, in which the blindfolded subject nulls his velocity with a joystick under the influences of a pseudorandom sled disturbance. Rotational VOR was measured at 0.3 and 0.8 Hz in the dark and the light. Static visual-vestibular interaction was tested with a standard rod and frame apparatus, while dynamic interaction was assessed by susceptibility to roll vection induced by a rotating peripheral visual field. Two examples are presented of how results from this study can aid in the interpretation of data from preflight/postflight testing of Space Shuttle/Spacelab crews on these same experiments.

Watt DGD, Money KE, Bondar RL, Thirsk RB, Garneau M, and Scully-Power P. Canadian medical experiments on shuttle flight 41-G. *Can Aeronaut Space J* 1985;31(3):215-226. During the 41-G mission, two payload specialist astronauts took part in six Canadian medical experiments designed to measure how the human nervous system adapts to weightlessness, and how this might contribute to space motion sickness. Similar tests conducted pre-flight provided base-line data, and post-flight experiments examined re-adaptation to the ground. No changes were detected in the vestibulo-ocular reflex during this 8-day mission. Pronounced proprioceptive illusions were experienced, especially immediately post-flight. Tactile acuity was normal in the fingers and toes, but the ability to judge limb position was degraded. Estimates of the locations of familiar targets were grossly distorted in the absence of vision. There were no differences in taste thresholds or olfaction. Despite pre-flight tests showing unusual susceptibility to motion sickness, the Canadian payload specialist turned out to be less susceptible than normal on-orbit. Re-adaptation to the normal gravity environment occurred within the first day after landing.

Watt DGD, Money KE, and Tomi LM. M.I.T./Canadian vestibular experiments on the Spacelab-1 mission: 3. Effects of prolonged weightlessness on a human otolith-spinal reflex. *Exp Brain Res* 1986;64:308-315. Reflex responses that depend on human otolith organ sensitivity were measured before, during and after a 10 day space flight. Otolith-spinal reflexes were elicited by means of sudden, unexpected falls. In weightlessness, "falls" were achieved using elastic cords running from a torso harness to the floor. Electromyographic (EMG) activity was recorded from gastrocnemius-soleus. The EMG response occurring in the first 100-120 ms of a fall, considered to be predominantly otolith-spinal in origin, decreased in amplitude immediately upon entering weightlessness, and continued to decline throughout the flight, especially during the first two mission days. The response returned to normal before the first post-flight testing session. The results suggest that information coming from the otolith organs is gradually ignored by the nervous system during prolonged space flight, although the possibility that otolith-spinal reflexes are decreased independent of other otolith output pathways cannot be ruled out.

Young LR. Tilted astronauts reveal the brain's balancing act. *New Sci* 1984.

Young LR, Oman CM, Watt DGD, Money KE, and Lichtenberg BK. Spatial orientation in weightlessness and readaptation to Earth's gravity. *Science* 1984;225:205-208. Unusual vestibular responses to head movements in weightlessness may produce spatial orientation illusions and symptoms of space motion sickness. An integrated set of experiments was performed during Spacelab 1, as well as before and after the flight, to evaluate responses mediated by the otolith organs and semicircular canals. A variety of measurements were used, including eye movements, postural control, perception of orientation, and susceptibility to space sickness.

Young LR, Shelhamer M, and Modestino S. M.I.T./Canadian vestibular experiments on the Spacelab-1 mission: 2. Visual vestibular tilt interaction in weightlessness. *Exp Brain Res* 1986;64:299-307. Adaptation to weightlessness includes the substitution of other sensory signals for the no longer appropriate graviceptor information concerning static spatial orientation. Visual-vestibular interaction producing roll circularvection was studied in weightlessness to assess the influence of otolith cues on spatial orientation. Preliminary results from four subjects tested on Spacelab-1 indicate that visual orientation effects were stronger in weightlessness than pre-flight. The rod and frame test of visual field dependence showed a weak post-flight increase in visual influence. Localized tactile cues applied to the feet in space reduced subjective vection strength.

CARDIOVASCULAR AND CARDIOPULMONARY

Arieli R, Boutellier U, and Farhi LE. Effect of water immersion on cardiopulmonary physiology at high gravity (+G_z). *J Appl Physiol* 1986;61(5):1686-1692. We compared the cardiopulmonary physiology of eight subjects exposed to 1, 2, and 3 G_z during immersion (35°C) to the heart level with control dry rides. Immersion should almost cancel the effects of gravity on systemic circulation and should leave the lung alone to gravitational influence. During steady-state breathing we measured ventilation, O₂ consumption (VO₂), CO₂ production, end-tidal PCO₂ (PACO₂), and heart frequency (f_H). Using CO₂ rebreathing techniques, we measured cardiac output, functional residual capacity, equivalent lung tissue volume, and mixed venous O₂ content, and we calculated arterial PCO₂ (PaCO₂). As G_z increased, ventilation, f_H, and VO₂ rose markedly, and PACO₂ and PaCO₂ decreased greatly in dry ride, but during immersion these variables changed very little in the same direction. Functional residual capacity was lower during immersion and decreased both the dry and immersed states as G_z increased, probably reflecting closure effects. Cardiac output decreased as G_z increased in dry rides and was elevated and unaffected by G_z during immersion. We conclude that most of the changes we observed during acceleration are due to the effect on the systemic circulation, rather than to the effect on the lung itself.

Arieli R, and Farhi LE. Gas exchange in tidally ventilated and non-steadily perfused lung model. *Respir Physiol* 1985;60:295-309. We studied the effect of cyclic lung perfusion - fast cycle in synchrony with heart beats and slow cycle in synchrony with ventilation - on gas exchange in a lung model. There was almost no effect in the fast cycle. In a homogenous single-lung unit, arterial PO₂ increased, and the (A-a)DO₂ decreased (by approximately 0.5 Torr), as the amplitude of the slow cyclic lung perfusion (TIP) increased. The calculated (A-a)DO₂ and (a-A)DCO₂ were negative. Maximal Pa_{O2} was found when peak lung perfusion was delayed with respect to ventilation by 0.2 of a cycle. In a non-homogeneous nine-unit lung, cyclic lung perfusion caused an increase in Pa_{O2} and a decrease in (A-a)DO₂ by 2 Torr as compared to steady perfusion. No apparent negative (A-a)DO₂ was found, but apparent negative (a-A)DCO₂ was calculated at no pulmonary shunt and also with 5% shunt. The correlation of cyclic lung perfusion to the reduced (A-a)DO₂ in dense-gas breathing - where large swings of pleural pressure are expected - and its effect on the diffusion capacity of the lung are discussed. Non-steady perfusion of the lung as caused by ventilatory movements expanded our understanding of gas exchange and shed some light on a few controversial experimental findings, such as the negative (a-A)DCO₂, the decreased (A-a)DO₂ while breathing dense gas, and the effects of gas density on diffusion capacity of the lung.

Arieli R, and Farhi LE. Gravity-induced hyperventilation is caused by a reduced brain perfusion. *Respir Physiol* 1987;69:237-244. The suggestion that hyperventilation caused by increased gravity is mediated by a decrease in brain perfusion has led us to propose a mathematical model based on: (1) the CO₂ balance equation for the respiratory center (RC), and (2) the relationship between RC blood flow (QRC), foot-to-head acceleration (G_z) and PRCCO₂, namely, $QRC = [1 - a(G_z - 1)](b \text{ PRCCO}_2 + c)$, where the coefficients a, b and c can be calculated from data in the literature. QRC is significantly affected by +G_z only at high PaCO₂. The model can be used to calculate oxygen pressure in the RC; the numbers so obtained are in good agreement with measurements of jugular vein PO₂ obtained by others.

Blomqvist CG. Orthostatic hypotension. In: CARDIOLOGY (Eds.) Parmley WW, and Chatterjee K. Philadelphia: J. B. Lippincott, 1990;1-12. Orthostatic hypotension is a common and sometimes disabling condition. Its pathophysiology has been studied extensively. Likely causes include many different defects that singly or in combination affect major mechanisms controlling blood flow, vascular resistance, arterial pressure, and intravascular volume. The control systems are complex, and their interactions are poorly understood. As a consequence, obvious and straight-forward therapeutic approaches often prove

ineffective, but seemingly paradoxical measures are sometimes helpful. These characteristics combine to make orthostatic hypotension a challenging topic. This review deals mainly with orthostatic hypotension occurring in the absence of structural neurological lesions.

Blomqvist CG. Orthostatic hypotension. *Hypertension* 1986;8(8):722-731.

Blomqvist CG, Gaffney FA, and Nixon JV. Cardiovascular responses to head-down tilt in young and middle-aged men. *Physiologist* 1983;26(6,Suppl):S81-S82.

Blomqvist CG, Nixon JV, Johnson RL, and Mitchell JH. Early cardiovascular adaptation to zero gravity simulated by head-down tilt. *Acta Astronautica* 1980;7(4/5):543-553. The early cardiovascular adaptation to zero gravity, simulated by head-down tilt at 5°, was studied in a series of 10 normal young men. The validity of the model was confirmed by comparing the results with data from Apollo and Skylab flights. Tilt produced a significant central fluid shift with a transient increase in central venous pressure, later followed by an increase in left ventricular size without changes in cardiac output, arterial pressure, or contractile state. The hemodynamic changes were transient with a nearly complete return to the control state within 6 hr. The adaptation included a diuresis and a decrease in blood volume, associated with ADH, renin and aldosterone inhibition.

Boutellier URS, Arieli R, and Farhi LE. Ventilation and CO₂ response during +G_z acceleration. *Respir Physiol* 1985;62:141-151. During foot-to-head acceleration (+G_z) ventilation increases despite a drop in alveolar Pco₂. In order to investigate the underlying mechanisms, we measured ventilation (VE), Vo₂, Vco₂ and PAco₂, cardiac output (Q) and mixed venous CO₂ concentration (Cvco₂) using non-invasive techniques in 5 subjects breathing either air or a gas mixture containing 5% CO₂ at +1, +2 and +3 G_z in a human centrifuge. Arterial Pco₂ was calculated from Fick's equation, using Cvco₂, Q and Vco₂. VE increased from 8.7 to 18.0 L/min during air breathing and from 19.6 to 36.9 L/min during CO₂ breathing at +1 and +3 G_z, respectively. The corresponding values for PAco₂ are 37.9 vs 26.9 Torr and 47.8 vs 46.4 Torr. Q dropped from 5.9 to 4.8 L/min during air breathing and remained the same during CO₂ breathing (6.7 vs 6.5 L/min). As the decrease of PAco₂ almost paralleled that of PAco₂, the arterio-alveolar CO₂ difference increased only slightly. The CO₂ response curve shifts gradually to the left with an increase in +G_z, a fact that does not support the hypothesis that foot-to-head acceleration increases CO₂ sensitivity.

Boutellier URS, and Farhi LE. Influence of breathing frequency and tidal volume on cardiac output. *Respir Physiol* 1986;66:123-133. The aim of our experiment was to investigate the influence of increasing either breathing frequency or tidal volume on cardiac output (Q), in normocapnia. We measured Q with a CO₂ rebreathing method in 6 men and 6 women in the sitting and the supine position, imposing different breathing patterns: in one set of experiments tidal volume was kept constant at 1 L while breathing frequency was randomly changed between 20, 30 and 40 breaths/min; in another breathing frequency was kept constant at 30 breaths/min while tidal volume was randomly altered between 1, 1.5 and 2L. Switching from open circuit breathing to rebreathing (for measurement of Q) required no change in breathing pattern. From the beginning, CO₂ was added to the inspired gas to maintain end-tidal Fco₂ 0.054, so as to obtain steady state conditions throughout the measurements. Q rose significantly when tidal volume was increased (938 ml/L rise in tidal volume when sitting, and 743 ml/L when supine). Breathing frequency had an insignificant effect (213 ml/10 breaths frequency increase when sitting and 142 ml/10 breaths when supine). The greater

influence of ventilation on Q when sitting than when supine is best explained by the fact that in the latter position venous return is already high. There are no demonstrable differences in this effect between males and females.

Boutellier URS, and Farhi LE. A fundamental problem in determining functional residual capacity or residual volume. *J Appl Physiol* 1986;60(5):1810-1813. To measure a lung volume that is not directly accessible, one often follows dilution of a single-gas tracer, present initially only in the lung or in a rebreathing bag. The final volume available to the tracer is assumed to be the sum of the two initial components. Since O_2 is taken up and CO_2 is eliminated during the few breaths required for mixing, the total volume changes. The error in lung volume due to this volume change can exceed 10%. In this paper we 1) present theoretical and experimental data to demonstrate the effect of CO_2 and O_2 exchange, 2) introduce a general equation, based on N_2 and Ar, which allows one to circumvent the problems created by these fluxes, and 3) show the pitfall of the back-extrapolation approach for a single tracer.

Buckey JC, Beattie JM, Gaffney FA, Nixon JV, and Blomqvist CG. Simplified right ventricular volume algorithm using one digitized view and transducer tilt angle. *Comput Cardiol* 1984;399-402. We recently described a technique for determining in-vitro right ventricular volume from multiple two-dimensional echocardiographic views taken at sequential angles. The product of sectional area and center of mass for each view (U) is integrated over the angle of tilt of the transducer to give volume. We now note that the plot of U vs. angle is almost triangular when the echoes are taken from the short axis position. The maximal U value (the vertex of the triangle) and the total angle span (the base) are then used in the equation (maximal U x total angle span)/2 to calculate volume. This new approximation provides an excellent correlation with actual volumes. We conclude that the triangular approximation provides accurate in-vitro estimates of right ventricular volume in normal human hearts.

Buckey JC, Beattie JM, Nixon JV, Gaffney FA, and Blomqvist CG. Right and left ventricular volumes in vitro by a new nongeometric method. *Am J Cardiac Imaging* 1987;1(3):227-233. We present an evaluation of a new nongeometric technique for calculating right and left ventricular volumes. This method calculates ventricular chamber volumes from multiple cross-sectional echocardiographic views taken from a single point as the echo beam is tilted progressively through the ventricle. Right and left ventricular volumes are calculated from both the approximate short axis and approximate apical position on 20 in vitro human hearts and compared with the actual chamber volumes. The results for both ventricles from both positions are excellent. Correlation coefficients are >0.95 for all positions; the standard errors are in the range of 5 to 7 mL and the slopes and intercepts for the regression lines are not significantly different from 1 and 0, respectively (except for the left ventricular short-axis intercept). For all positions, approximately 6 to 8 views are needed for peak accuracy (7.5° to 10° separation). This approach offers several advantages. No geometric assumptions about ventricular shape are made. All images are acquired from a single point (or window), and the digitized points can be used to make a three-dimensional reconstruction of the ventricle. Also, during the calculations a volume distribution curve for the ventricle is produced. The shape of this curve can be characteristic for certain situations (ie, right ventricle, short axis) and can be used to make new simple equations for calculating volume. We conclude that this is an accurate nongeometric method for determining both right and left ventricular volumes in vitro.

Buckey JC, Goble RL, and Blomqvist CG. A new device for continuous ambulatory central venous pressure measurement. *Med Instrum* 1987;21(4):238-243. We have developed a device for continuous direct measurement of human central venous pressure (CVP) during space flight. Normal resting CVP is typically in the range of 5-10 mm Hg; in zero gravity, the expected changes are ± 5 mm Hg or less. A 1 mmHg change in CVP can represent a substantial intravascular fluid shift. The device is small, battery powered, and designed to run for a least 24 hours. Pressure is measured in a saline solution-filled catheter inserted into a central vein. The transducer is placed in the axilla at the level of the catheter tip to offset hydrostatic gradients. A pump and an electronic system mount on the leg. This assembly provides a slow, continuous infusion of heparinized saline solution to maintain the patency of the catheter. The electronic system generates a digital display in mm Hg, an analog output, and a visible and audible alarm for excessive pressure. An air-filled syringe allows for a two-point calibration (zero and a positive pressure generated by measured compression of a known gas volume). A two-failure tolerant system minimizes electric shock hazards. Two latex diaphragms separate the saline solution from the transducer surface, and the electronic system and pump chamber are in separate enclosures. A clear polycarbonate case allows bubbles to be seen. The unit has been tested for pump function, temperature stability, drift, and accuracy. We conclude that this approach provides a unit with sufficient stability, accuracy, and temperature insensitivity for measuring ambulatory CVP for up to 28 hours. The design may be suitable for ambulatory measurement of other intravascular and intracardiac pressures.

Buckey JC, Peshock RM, and Blomqvist CG. Deep venous contribution to hydrostatic blood volume change in the human leg. *Am J Cardiol* 1988;62:449-453. The causes of orthostatic intolerance following prolonged bed rest, head-down tilt or exposure to zero gravity are not completely understood. One possible contributing mechanism is increased venous compliance and peripheral venous pooling. The present study attempted to determine what proportion of the increased calf volume during progressive venous occlusion is due to deep venous filling. Deep veins in the leg have little sympathetic innervation and scant vascular smooth muscle, so their compliance may be determined primarily by the surrounding skeletal muscle. If deep veins make a large contribution to total leg venous compliance, then disuse-related changes in skeletal muscle mass and tone could increase leg compliance and lead to decreased orthostatic tolerance. The increase in deep venous volume during progressive venous occlusion at the knee was measured in 6 normal subjects using calf cross-sectional images obtained with magnetic resonance imaging. Conventional plethysmography was used simultaneously to give an independent second measurement of leg volume and monitor the time course of the volume changes. Most of the volume change at all occlusion levels (20, 40, 60, 80 and 100 mm Hg) could be attributed to deep venous filling (90.2% at 40 mm Hg and 50.6% at 100 mm Hg). It is concluded that a large fraction of the calf volume change during venous occlusion is attributable to filling of the deep venous spaces. This finding supports theories postulating an important role for physiological mechanisms controlling skeletal muscle tone during orthostatic stress.

Buckey JC, Sweeney FM, Kim LT, Beattie JM, Nixon JV, Gaffney FA, and Blomqvist CG. Stroke volume in-vivo using multiple 2D echo views from one echo window. *Comput Cardiol* 1985;293-296. We recently validated in-vitro a new mathematical approach to echocardiographic volume calculation. With this method all echo views are acquired from one point as the transducer is tilted. The angle of tilt of the transducer must be measured. A tilt frame was designed for use in-vivo that does not interfere with imaging. The method was then tested in-vivo by comparing echocardiographic stroke volume with stroke volume determined by the acetylene rebreathing technique. Normal subjects were studied with either lower body negative pressure or continuous isoproterenol infusion. The regression line was $\text{Echo SV} = -19.7 + 1.2 \cdot \text{Acet SV}$, with $R=0.80$, $\text{SEE}=17.1$, $\text{MPE}=17\%$. We conclude that this is an accurate, non-geometric method for ventricular volume calculation.

Buckey JC, Watenpaugh DE, Kim LT, Smith ML, Gaffney FA, and Blomqvist CG. Initial experience with a new plethysmograph for zero-g use. *Physiologist* 1985;28(6,Suppl):S145-S146. We developed and tested a system for venous occlusion plethysmography (SVOP) suited for use in Spacelab. This unit measures changes in limb circumference by using an optical shaft encoder connected to a band encircling the limb. Circumference changes are converted to digital pulses and displayed. Flow is then calculated in ml/min/100 ml. This instrument was compared to mercury-in-silastic (Whitney) gauges at rest and with hyperemia after 3 min of arterial occlusion. Overall the correlation coefficient was 0.95, $SVOP = 0.90 \times \text{Whitney} + 0.44$; at rest alone the correlation coefficient was 0.89, $SVOP = 0.62 \times \text{Whitney} + 0.75$. We conclude the SVOP produces good flow data, needs no calibration, and contains no toxic materials making it suitable for zero-G use.

Convertino VA, Doerr DF, Eckberg DL, Fritsch JM, and Vernikos-Danellis J. Carotid baroreflex response following 30 days exposure to simulated microgravity. *Physiologist* 1989;32(1,Suppl):S67-S68.

Fritsch JM, Rea RF, and Eckberg DL. Carotid baroreflex resetting during drug-induced arterial pressure changes in humans. *Am J Physiol* 1989;256(25):R549-R553. We studied human baroreflex resetting during 25 min of drug-induced arterial pressure changes in 10 healthy volunteers. Average (\pm SE) base-line systolic pressure of 113 ± 4 fell to 102 ± 3 during nitroprusside infusions and rose to 135 ± 6 mmHg during phenylephrine infusions. Average base-line R-R intervals of 932 ± 37 shortened to 820 ± 39 during nitroprusside infusions and lengthened to $1,251 \pm 61$ ms during phenylephrine infusions. Carotid baroreceptor-cardiac reflex responses were evaluated with a complex series of neck chamber pressure changes, and R-R intervals were plotted as functions of carotid distending pressure. Baroreceptor-cardiac reflex relations shifted on both R-R interval and arterial pressure axes during drug infusions, but there was no significant change of the maximum slope or range of R-R interval responses. The position of baseline R-R intervals on the reflex relation (operational point) changed significantly. Resting R-R intervals were closer to threshold during pressure reductions and closer to saturation for baroreceptor-cardiac responses during pressure elevations. These results document short-term partial resetting of human baroreceptor-cardiac reflex responses as early as 25 minutes after the onset of arterial pressure changes.

Gaffney FA, Bastian BC, Thal ER, Atkins JM, and Blomqvist CG. Passive leg raising does not produce a significant or sustained autotransfusion effect. *J Trauma* 1982;22(3):190-193. Passive leg raising is widely used to treat hypotension associated with hypovolemia. Presumably gravity causes a central translocation of leg venous blood and an increase in filling pressure, cardiac output, and arterial pressure. Ten healthy volunteers, 25 to 35 years old, had measurements of heart rate, blood pressure, and cardiac output in the supine position after 20 sec and 7 min of 60° passive leg elevation. The protocol was performed 3 and 45 min after the subjects changed from an ambulatory upright to a supine position. Stroke volume and cardiac output increased transiently (8-10%) when the legs were raised after 3 min rest in the supine position. By 7 min of leg elevation, these beneficial effects disappeared. After 45 min supine, leg raising had no effect on stroke volume or cardiac output but increased blood pressure (4 mm Hg) by increasing peripheral resistance (15%). Thus, leg raising, like application of the MAST trousers, fails to produce any sustained increase in cardiac output or stroke volume. Small venous leg volumes and time-dependent changes in the distribution of venous volume and compliance may explain the absence of any sustained 'autotransfusion' effect.

Gaffney FA, Lane LB, Pettinger W, and Blomqvist CG. Effects of long-term clonidine administration on the hemodynamic and neuroendocrine postural responses of patients with dysautonomia. *Chest* 1983;83S(Feb,Suppl):S436-S438. Patients with mitral valve prolapse syndrome (MVPS), vasoregulatory asthenia, and poor postural adjustment often have orthostatic intolerance characterized by tachycardia and a narrow pulse pressure on standing. Autonomic dysfunction is thought to play an important role. Increased α -adrenergic activity has been shown in MVPS patients with orthostatic intolerance. We measured hemodynamic and neuroendocrine responses to long-term oral clonidine therapy in eight women, aged 36 ± 1.8 years (27 to 44 years). None had responded favorably to β -blockers. Heart rate, blood pressure, oxygen consumption, cardiac output, and plasma norepinephrine levels were measured in both supine and standing positions, before and after one to four weeks of clonidine (0.3 to 0.4 mg daily). Clonidine reduced standing plasma norepinephrine levels, total peripheral resistance, and diastolic blood pressure; a smaller decrease in cardiac output on standing was noted. Plasma volumes increased 12 percent. Mild reductions in plasma catecholamines and total peripheral resistance are associated with fewer, not more, orthostatic symptoms in this group of patients. "Placebo" or mild sedative effects may explain part of the response to clonidine, but the hemodynamic and neuroendocrine data suggest that decreased α -adrenergic hyperactivity may also be important.

Gaffney FA, Nixon JV, Karlsson ES, Campbell W, Dowdey ABC, and Blomqvist CG. Cardiovascular deconditioning produced by 20 hours of bedrest with head-down tilt (-5°) in middle-aged healthy men. *Am J Cardiol* 1985;56:634-638. Cardiovascular deconditioning after prolonged bedrest has been attributed to inactivity. To examine the role of the altered distribution of body fluid, 5 healthy men, aged 41 to 48 years, were studied before, during and after a 20-hour period of bedrest with head-down tilt (-5°). This intervention produces a marked central shift of intravascular and interstitial fluid, but the short duration minimizes the effects of inactivity. Central venous pressure, cardiac output and stroke volume all increased significantly ($p < 0.05$) from supine baseline mean values; central venous pressure from 8.6 to 12.6 cm H_2O , cardiac output from 6.9 to 7.9 liters/min, and stroke volume from 104 to 113 ml after 15 minutes of tilt, but all values returned to baseline within 20 hours. Supine central venous pressure after tilt was 7.4 cm H_2O , cardiac output 5.7 liters/min and stroke volume 84 ml. Blood volume decreased 0.51 liters. After tilt, orthostatic stress produces a higher heart rate (90 ± 18 vs 68 ± 12 beats/min). Maximal oxygen consumption decreased (2.36 ± 0.41 vs 2.62 ± 0.48 liters/min), mainly owing to reduced stroke volume (87 ± 22 vs 107 ± 18 ml, $p < 0.05$). Thus, tilt produced a transient increase in central venous pressure, stroke volume and cardiac output, but supine mean values were below baseline levels after 20 hours. The post-tilt state was qualitatively and quantitatively similar to that seen after 2 to 3 weeks of bedrest or several days of spaceflight. These results are also similar to those from a previously studied group of ten 20- to 30-year-old normal men. However, the increase in central venous pressure tended to be larger and of longer duration in the middle-aged group. Furthermore, in the young men the initial increase in stroke volume produced relative bradycardia, with no change in cardiac output and arterial pressure. Cardiac output increased in the middle-aged group, but arterial pressure was controlled by vasodilatation. Heart rate did not change. The results support the concept that cardiovascular deconditioning after bedrest is primarily an adaptation to a postural fluid shift rather than to inactivity. Age-related differences in hemodynamic responses to central fluid shifts appear to be present.

Gaffney FA, Thal ER, Taylor WF, Bastian BC, Weigelt JA, Atkins JM, and Blomqvist CG. Hemodynamic effects of medical anti-shock trousers (MAST garment). *J Trauma* 1981;21(11):931-937. Despite widespread use of the Medical Anti-Shock Trousers (MAST) little is known about the exact mechanism by which they increase arterial pressure. It is assumed that an autotransfusion occurs. To examine this question, blood pressure, heart rate, forearm blood flow, cardiac output, and stroke volume were measured in ten healthy adults, supine and during 60° headup tilt with MAST garment pressures of 40 and

100 mm Hg. Supine, the garment produced no *net* 'autotransfusion,' but raised blood pressure (27%) by increasing peripheral resistance (48%) with decreased stroke volume and cardiac output (18%). During headup tilt without the MAST device, venous pooling in the legs decreased stroke volume (52%), cardiac output (30%), and increased total peripheral resistance (40%). Application of the garment during tilt shifted this blood centrally, producing increased stroke volume (14%). In supine normovolemic subjects, the garment raised pressure almost exclusively by increased systemic afterload. Forearm vascular resistance did not change and the increased pressure augmented flow to the arm, i.e., to noncompressed tissue. With increased venous pooling during tilt, the MAST garment acted as a 'G-suit' and caused a central shift of blood volume. These findings could explain: 1) why fluid replacement is not always adequate to maintain pressure when deflating the trousers; 2) why the trousers should not be used if one wished to avoid increasing afterload (e.g., certain patients with acute myocardial infarction). We conclude that the MAST garment acts as a local, effective, nonpharmacologic vasoconstrictor and should be used when such an effect is clinically appropriate.

Guy HJ, Gaines RA, Hill PM, Wagner PD, and West JB. Computerized, noninvasive tests of lung function. A flexible approach using mass spectrometry. *Am Rev Respir Dis* 1976;113:737-744. The design, operation, and some applications of a computerized pulmonary function testing system built around a mass spectrometer are described. The test sequence, performed in 10 to 20 min, includes spirometry, a single-breath N_2 washout, and measurement of the diffusing capacity of the lung for CO. Secondary tests, an integral part of the sequence, include rebreathing estimates of lung volume and cardiac output, and a breath-by-breath analysis of over-all gas exchange. These secondary tests lead to computer modeling of a one-compartment lung closely matched to the subject's lungs. Differences between alveolar plateau slopes in the model and real lung provide information about the degree of ventilation-perfusion mismatch in the subject. It is expected that the combination of tests will be useful in the early detection of lung disease.

Kasting GA, Eckberg DL, Fritsch JM, and Birkett CL. Continuous resetting of the human carotid baroreceptor-cardiac reflex. *Am J Physiol* 1987;252:R732-R736. Although human baroreflex responses have been studied during night as well as day, there has been no attempt to distinguish circadian changes of baroreflex function from those related to sleep. We measured carotid baroreceptor-cardiac reflex responses serially during a 24-h period in 11 normotensive volunteers who were awake and cooperative during testing. We applied sequences of ramped R-wave triggered neck chamber pressure changes from +40 to -65 mmHg, during held expiration, at 3-h intervals. Subjects maintained their usual sleep-wake cycles but were awakened for three 30-min periods for night testing. There was no systematic change of baroreflex slope during the 24-h period. There were, however, parallel shifts of the entire sigmoid baroreceptor-cardiac reflex response relation along its R-R interval and arterial pressure axes associated with small, but significant, circadian changes of baseline R-R intervals and arterial pressures. Thus, although our data do not point toward major circadian variability of baroreflex responsiveness, they provide evidence for an ongoing process of human baroreflex resetting.

Michels DB, and West JB. Distribution of pulmonary ventilation and perfusion during short periods of weightlessness. *J Appl Physiol* 1978;45(6):987-998. Information on the distributions of pulmonary ventilation and perfusion was obtained from four subjects on board a Learjet during 112 weightless periods lasting up to 27 s each. Zero gravity (G) was obtained during all or part of each test by varying the aircraft flight profile. Single-breath N_2 washouts were performed with the test inspiration containing an initial bolus of argon at residual volume (RV). When the test inspiration was at 0 G, and the washout at 0 G or greater, the terminal rises and the cardiogenic oscillations in both N_2 and argon were small and often absent. If instead the test inspiration was at 1 G with the washout at 0 G, the terminal rises were again small or absent but the

cardiogenic oscillations remained. The terminal rise and the cardiogenic oscillations for N_2 , but not argon, were also nearly eliminated by performing just the preliminary exhalation to RV at 0 G with the test inspiration and washout following at 1 G. Alveolar plateaus for N_2 sloped upward at 0 G apparently due to nontopographical inequalities of ventilation. In further tests during air breathing, recordings were made of expired partial pressure of oxygen (PO_2) and carbon dioxide (PCO_2) following a brief hyperventilation and a 15-s breath hold. These recordings revealed marked cardiogenic oscillations in PO_2 and PCO_2 at 1 G that were enhanced at 2 G but almost eliminated at 0 G. The results suggest that virtually all the topographical inequality of ventilation, blood flow, and lung volume seen under 1-G conditions are abolished during short periods of 0 G.

Nichol GM, Michels DB, and Guy HJB. Phase V of the single-breath washout test. *J Appl Physiol* 1982;52(1):34-43. A downward-deflecting phase V is often seen following the phase IV terminal rise in the single-breath N_2 washout test (SB N_2). This phase V was studied in eight normal nonsmoking subjects aged 27-41, using both the SB N_2 test and single-breath washouts of boluses of inert tracer gas slowly inhaled from residual volume (RV). All of the subjects showed a distinct phase V in both tests. Expiratory flow rates between 0.1 and 2.0 l/s were used; at each flow rate phase V appeared shortly after expiration became flow limited. Thus the volume above RV at which phase V began increased with increasing expiratory flow rate. The difference between the exhaled volumes at which flow became limited and phase V appeared was shown to be approximately equal to the anatomic dead space. This behavior is predicted by a model of lung emptying in a gravitational field. As expiration proceeds, flow limitation occurs first in the (tracer-poor) lower lung regions and then progresses toward the (tracer-rich) upper lung regions causing phase IV. When all lung regions have finally become flow limited, the amount of flow from the upper regions decreases relative to that of the lower regions, thereby causing phase V.

Nixon JV, Murray RG, Bryant C, Johnson RL, Mitchell JH, Holland OB, Gomez-Sanchez C, Vergne-Marini P, and Blomqvist CG. Early cardiovascular adaptation to simulated zero gravity. *J Appl Physiol* 1979;46(3):541-548. Physiological responses characterizing the early adaptation to weightlessness were studied in five normal men. Supplementary data on central venous pressure (CVP) were obtained in three additional subjects. Zero gravity was simulated by a 24-h period of head-down tilt at 5°. Tilt produced a central fluid shift. Orthostatic tolerance and exercise capacity were reduced posttilt. These changes were similar to those observed during and after space flight and support the validity of the experimental model. CVP increased transiently from 5.6 to a peak of 8.5 cmH₂O ($P < 0.02$). Control levels for CVP were approached at 90 min, at that time the echocardiographic left ventricular end-diastolic diameter reached a maximum (4.7 cm, control 3.9 cm, $P < 0.05$). There were no changes in arterial pressure, cardiac output, or left ventricular contractile state. Urine flow was 1.98 ml·min⁻¹ during the initial 8 h compared to 1.36 during the final 16 h ($P < 0.05$). Blood volume decreased by 0.5 liter ($P < 0.05$). Plasma renin activity, aldosterone, and antidiuretic hormone were depressed initially but returned to base line within 24 h. Plasma electrolytes remained unchanged. The results suggest that hemodynamic adaptation occurs rapidly and is essentially accomplished by 6 h. Adaptation includes a diuresis and reduction in blood volume.

Nixon JV, Saffer SI, Lipscomb K, and Blomqvist CG. Three-dimensional echoventriculography. *Am Heart J* 1983;106(3):435-443. A method of generating a three-dimensional image of the human left ventricle by computer techniques is described. The volume of each image was estimated by a modification of Simpson's rule. The method was applied to nine suitable patients and estimations of end-diastolic and end-systolic volumes were compared to volumes determined by cineangiography. Significant linear correlation coefficients of 0.95 and 0.94 were obtained for end-diastolic and end-systolic volumes,

respectively. The standard errors of estimate were 9 ml for end-diastolic volumes and 7 ml for end-systolic volumes. The value of this methodology lies in the ability to estimate left ventricular volumes with accuracy, using an imaging technique of little inconvenience and no risk to the patient and computer hardware that is readily available at most clinical institutions.

Parra B, Buckey J, DeGraff D, Gaffney FA, and Blomqvist CG. Echocardiographic measurements of left ventricular mass by a non-geometric method. *Aviat Space Environ Med* 1987;58(9,Suppl):A64-A68. The accuracy of a new non-geometric method for calculating left ventricular myocardial volumes from 2-D echocardiographic images was assessed *in vitro* using 20 formalin-fixed normal human hearts. Serial oblique short axis images were acquired from one point at 5° intervals, for a total of 10 to 12 cross sections. Echocardiographic myocardial volumes were calculated as the difference between the volumes defined by the epi- and endo-cardial surfaces. Actual myocardial volumes were determined by water displacement. Volumes ranged from 80 to 174 ml (mean 130.8 ml). Linear regression analysis demonstrated excellent agreement between echocardiographic (X) and direct measurements (Y) i.e., $y = 0.98x + 4.3$ ml; $r = 0.94$; SEE = 8.4; $p = 0.001$. Comparison of 10 duplicate measurements by two independent observers yielded an r of 0.96. These *in vitro* results suggest that with this technique, quantitative analysis of a limited number of cross sectional echocardiographic views will provide accurate left ventricular mass estimates.

Poliner LR, Dehmer GJ, Lewis SE, Parkey RW, Blomqvist CG, and Willerson JT. Left ventricular performance in normal subjects: a comparison of the responses to exercise in the upright and supine positions. *Circulation* 1980;62(3):528-534. Left ventricular (LV) performance at rest and during multi-level exercise in the supine and upright positions was studied in seven normal subjects with equilibrium radionuclide ventriculography. The mean left ventricular end-diastolic volume (LVEDV) during supine rest was 107 ± 10 ml (\pm SEM) and 85 ± 6 ml ($p < 0.02$) in the upright position; the mean resting left ventricular end-systolic volumes (LVESV) were not different in the upright and supine positions. The LV ejection fraction (LVEF) tended to be slightly higher in the supine ($76 \pm 2\%$) than in the upright position ($72 \pm 4\%$). The resting heart rate was 89 ± 5 beats/min upright, compared with 71 ± 6 beats/min supine ($p < 0.05$). Multilevel exercise testing was carried out at a low work load of 300 kpm/min, an intermediate work load of 600-750 kpm/min and a peak work load of 1092 ± 66 kpm/min supine and 946 ± 146 kpm/min upright ($p < 0.05$). With peak exercise, supine LVEDV increased significantly, to 135 ± 13 ml (27%), but LVESV did not change. LVEF increased from $76 \pm 2\%$ to $84 \pm 2\%$ ($p < 0.05$). With upright exercise, LVEDV increased 39% above the resting level, to 116 ± 8 ml ($p < 0.02$), but remained lower than the supine LVEDVs at intermediate ($p < 0.05$) and peak work loads. LVESV decreased significantly by 41%, to 19 ± 3 ml, and was significantly smaller than the corresponding supine volume at intermediate and peak exercise ($p < 0.05$). LVEF increased from $72 \pm 4\%$ to $91 \pm 2\%$ ($p < 0.05$), which was significantly higher than peak supine LVEF ($p < 0.05$). Heart rates at rest and during exercise were higher in the upright position ($p < 0.05$), but arterial pressures and double products did not differ significantly.

Measurements of LV volumes at rest and during exercise in both the supine and upright positions by dynamic radionuclide scintigraphy suggest that stroke volume during exercise is maintained by a combination of the Frank-Starling mechanism and an enhanced contractile state.

Raven PB, Pape G, Taylor WF, Gaffney FA, and Blomqvist CG. Hemodynamic changes during whole body surface cooling and lower body negative pressure. *Aviat Space Environ Med* 1981;52(7):387-391. Six young healthy male subjects were studied to evaluate the use of whole body surface cooling (WBSC) as an antiorthostatic intervention. Previous studies in our laboratory have demonstrated that perfusion of an Apollo cooling garment with 16°C water produced a significant increase in stroke volume and decrease in

heart rate at rest and during lower body negative pressure (LBNP). However, optimal perfusion temperatures have not been determined. The present study examined the effects of WBSC using perfusion of water at a temperature of 10°C. This perfusion temperature produced a greater decrease in mean skin temperature (T_{sk}) than water at 16°C, -4°C drop compared to -2°C respectively. The hemodynamic effects were also more prominent with 10°C water as shown by the increase in stroke volume of 11% at rest and of 35% during LBNP at -50 torr compared to control measurements at ambient temperature. Heart rates were lowered significantly (8 beats/min) and systolic arterial blood pressure was higher (8 torr). Cooling with 10°C water produced a slight increase in muscle tone, reflected by a small but significant increase (+84 ml/min) in oxygen uptake. These data suggest that WBSC is an effective nonpharmacologic means of controlling preload and deserves further investigation as an antiorthostatic intervention.

Raven PB, Rohm-Young D, Blomqvist CG. Physical fitness and cardiovascular response to lower body negative pressure. *J Appl Physiol* 1984;56(1):138-144. Fourteen young male volunteers (mean age 28.1 yr) underwent maximal exercise performance testing and lower body negative pressure (LBNP) challenge to -50 Torr. Two distinct groups, fit (F, $n = 8$), mean maximal aerobic capacity (VO_{2max}) = 70.2 ± 2.6 (SE) ml O_2 $kg^{-1} \cdot min^{-1}$, and average fit (AF, $n = 6$), mean VO_{2max} = 41.3 ± 2.9 ml O_2 $kg^{-1} \cdot min^{-1}$, $P < 0.001$, were evaluated. Rebreathing CO_2 cardiac outputs, heart rate (HR), blood pressure (BP), and leg circumference changes were monitored at each stage of progressive increases in LBNP to -50 Torr. The overall hemodynamic responses of both groups of subjects to LBNP were qualitatively similar to previous findings. There were no differences between F and AF in peripheral venous pooling as shown by a leg compliance (Δ leg volume/ Δ LBNP) for the F of 12.6 ± 1.1 and for the AF 11.6 ± 2.0 , $P > 0.05$. The F subjects had significantly less tachycardic response [Δ HR/ Δ systolic BP of F = 0.7 beats/Torr] to LBNP to -50 Torr than the AF subjects [Δ HR/ Δ systolic BP of unfit (UF) = 1.36 beats/Torr], $P < 0.05$. In addition, overall calculated peripheral vascular resistance was significantly higher in the AF subjects ($P < 0.001$), and there was a more marked decrease in systolic BP of the F subjects between the LBN pressures of -32 to -50 Torr. We concluded that the reflex response to central hypovolemia was altered by endurance exercise training.

Raven PB, Saito M, Gaffney FA, Schutte J, and Blomqvist CG. Interactions between surface cooling and LBNP-induced central hypovolemia. *Aviat Space Environ Med* 1980;51(5):497-503. The interaction between whole body surface cooling (WBSC) and progressive lower body negative pressure (LBNP) to -50 torr was evaluated in nine healthy male volunteers, mean age 29 ± 1.7 years. WBSC, accomplished by circulating 16°C water through an Apollo cooling garment, produced a significant drop in mean skin temperature of 1.96°C ($p < 0.001$). Cardiac output (Q) was measured by the C_2H_2 rebreathing technique. Changes in leg volume (LgV) were monitored by a Whitney strain gauge. WBSC at rest produced a significant decrease in leg volume of 0.27 l ($p < 0.01$). Heart rate decreased (-7 bpm, $p < 0.01$) and systolic arterial blood pressure was increased (+6 torr, $p < 0.02$). The hemodynamic effects of cooling were maintained throughout progressive levels of LBNP with consistently lower leg volumes and heart rates and higher stroke volumes and systolic pressure ($p < 0.01$ for all measurements). The data suggest that WBSC produces a central displacement of cutaneous venous volume resulting in an increase in stroke volume.

Roberts LA, Slocum GR, and Riley DA. Morphological study of the innervation pattern of the rabbit sinoatrial node. *Am J Anat* 1989;185:74-88. The pattern of nerves, ganglia, and fine nerve processes in the adult rabbit sinoatrial node, identified by microelectrode recording was defined by staining histochemically for cholinesterase followed by silver impregnation. A generalized repeatable pattern of innervation was recognized, including 1) a large ganglionic complex inferior to the sinoatrial node; 2) two or three moderately large nerves traversing the sinoatrial node parallel to the crista terminalis; 3) nerves entering the region from the atrial septum, the superior vena cava, and the inferior vena cava; and 4) a fine network of

nerve processes, particularly extensive in the morphologically dense small-cell part of the sinoatrial node. When the site of initial depolarization in the node was located and marked by a broken-off electrode tip, it was found, after cholinesterase staining, to be characterized by a cluster of cells enclosed in a nest or basket of fine nerves. Similar nested cell clusters were observed elsewhere in the sinoatrial node in this same preparation and in other hearts. A complex interweaving of atrial muscle fibers was observed medial and inferomedial to the sinoatrial node, which may form the anatomical basis for the lack of conduction through this region. The morphological pattern of nerves, ganglia, and myocardial cells described in this study emphasizes the complexity of innervation of the sinoatrial node, including its intrinsic neural elements. Cholinesterase/silver staining can be useful in the definition and comparison of electrophysiologically identified sites within the sinoatrial node.

Shykoff BE, and Swanson HT. A model-free method for mass spectrometer response correction. *J Appl Physiol* 1987;63(5):2148-2153. A new method for correction of mass spectrometer output signals is described. Response-time distortion is reduced independently of any model of mass spectrometer behavior. The delay of the system is found first from the cross-correlation function of a step change and its response. A two-sided time-domain digital correction filter (deconvolution filter) is generated next from the same step response data using a regression procedure. Other data are corrected using the filter and delay. The mean squared error between a step response and a step is reduced considerably more after the use of a deconvolution filter than after the application of a second-order model correction. O_2 consumption and CO_2 production values calculated from data corrupted by a simulated dynamic process return to near the uncorrupted values after correction. Although a clean step response or the ensemble average of several responses contaminated with noise is needed for the generation of the filter, random noise of magnitude $\leq 0.5\%$ added to the response to be corrected does not impair the correction severely.

Snell PG, Martin WH, Buckey JC, and Blomqvist CG. Maximal vascular leg conductance in trained and untrained men. *J Appl Physiol* 1987;62(2):606-610. Lower leg blood flow and vascular conductance were studied and related to maximal oxygen uptake in 15 sedentary men (28.5 ± 1.2 yr, mean \pm SE) and 11 endurance-trained men (30.5 ± 2.0 yr). Blood flows were obtained at rest and during reactive hyperemia produced by ischemic exercise to fatigue. Vascular conductance was computed from blood flow measured by venous occlusion plethysmography, and mean arterial blood pressure was determined by auscultation of the brachial artery. Resting blood flow and mean arterial pressure were similar in both groups (combined mean, $3.0 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ ml}^{-1}$ and 88.2 mmHg). After ischemic exercise, blood flows were 29- and 19-fold higher ($P < 0.001$) than rest in trained ($83.3 \pm 3.8 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ ml}^{-1}$) and sedentary subjects ($61.5 \pm 2.3 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ ml}^{-1}$), respectively. Blood pressure and heart rate were only slightly elevated in both groups. Maximal vascular conductance was significantly higher ($P < 0.001$) in the trained compared with the sedentary subjects. The correlation coefficients for maximal oxygen uptake vs. vascular conductance were 0.81 (trained) and 0.45 (sedentary). These data suggest that physical training increases the capacity for vasodilation in active limbs and also enables the trained individual to utilize a larger fraction of maximal vascular conductance than the sedentary subject.

Sprenkle JM, Eckberg DL, Goble RL, Schelhorn JJ, and Halliday HC. Device for rapid quantification of human carotid baroreceptor-cardiac reflex responses. *J Appl Physiol* 1986;60(2):727-732. We designed, constructed, and evaluated a new device to characterize the human carotid baroreceptor-cardiac reflex response relation rapidly. We designed this system for study of reflex responses of astronauts before, during, and after space travel. The system comprises a new tightly sealing silicone rubber neck chamber, a stepping motor-driven electro-deposited nickel bellows pressure system, capable of delivering sequential

R-wave-triggered neck chamber pressure changes between +40 and -65 mmHg, and a microprocessor-based electronics system for control of pressure steps and analysis and display of responses. This new system provokes classic sigmoid baroreceptor-cardiac reflex responses with threshold, linear, and saturation ranges in most human volunteers during one held expiration.

Tomioka S, Kubo S, Guy HJB, and Prisk GK. Gravitational independence of single-breath washout in recumbent dogs. *J Appl Physiol* 1988;64(2):642-648. To examine the mechanisms of lung filling and emptying, Ar-bolus and N₂ single-breath washout tests were conducted in 10 anesthetized dogs (prone and supine) and in three of those dogs with body rotation. Transpulmonary pressure was measured simultaneously, allowing identification of the lung volume above residual volume at which there was an inflection point in the pressure-volume curve (V_{IP}). Although phase IV for Ar was upward, phase IV for N₂ was small and variable, especially in the prone position. No significant prone to supine differences in closing capacity for Ar were seen, indicating that airway closure was generated at the same lung volumes. The maximum deflections of phase IV for Ar and N₂ from extrapolated phase III slopes were smaller in the prone position, suggesting more uniform tracer gas concentrations across the lungs. V_{IP} was smaller than the closing volume for Ar, which is consistent with the effects of well-developed collateral ventilation in dogs. Body rotation tests in three dogs did not generally cause an inversion of phase III or IV. We conclude that in recumbent dogs regional distribution of ventilation is not primarily determined by the effect of gravity, but by lung, thorax, and mediastinum interactions and/or differences in regional mechanical properties of the lungs.

West JB, Guy HJ, Gaines RA, Hill PM, and Wagner PD. Battery of single-breath tests for rapid measurement of pulmonary function using a respiratory mass spectrometer. *Pneumologie* 1975;151(4):258-263. Modern respiratory mass spectrometers are much improved with respect to their reliability and flexibility. In addition, small dedicated computers are now available for the rapid analysis of data. We describe here a package of single-breath tests utilizing a respiratory mass spectrometer, flow and volume meters, and a small dedicated computer which allows a considerable amount of information about pulmonary function to be obtained in a non-invasive manner in a short time. The principle of the measurement is that the patient performs a series of respiratory maneuvers in approximately 10 min and all data analysis is done by computer. Such a battery of single-breath tests might be valuable in a general hospital setting for the screening of large numbers of patients.

The initial stimulus for investigating a series of single breath tests was provided by the National Aeronautics and Space Administration who were interested in a non-invasive series of lung function tests which could be used to measure the pulmonary function of orbiting astronauts. The package that I am about to describe was originally developed for this purpose and we hope it will fly in the shuttle at the end of this decade. However, the battery has also been set up in one of the University hospitals where it is being used to collect data on patients with minimal amounts of lung disease.

RENAL AND ENDOCRINE

Leach CS. Fluid control mechanisms in weightlessness. *Aviat Space Environ Med* 1987;58(9,Suppl):A74-A79. Experiments performed on Space Shuttle flights have emphasized study of the earliest effects of the cephalad fluid shift resulting from microgravity. Analysis of one subject's urine collected during flight showed that a sharp increase in antidiuretic hormone occurred within 2 h of launch, followed by an increase in cortisol excretion. Although this subject had symptoms of the space adaptation syndrome (SAS), inflight data from Spacelab missions suggested that these transient changes were not caused by SAS. Unpaired *t*-tests and Mann-Whitney tests showed that before and after flight, plasma thyroxine and urine osmolality were significantly higher in Shuttle crewmembers who exhibited more severe symptoms of SAS than in asymptomatic crewmembers. Collection of inflight data from more crewmembers should allow distinction between the effects of SAS and effects of weightlessness, and in the future several additional fluid regulation hormones will be measured in samples from crewmembers for a more complete understanding of fluid control during weightlessness.

Leonard JI. Understanding metabolic alterations in space flight using quantitative models: fluid and energy balance. *Acta Astronautica* 1986;13(6/7):441-457. This report summarizes many of the results obtained during the Skylab program, on metabolic changes during weightlessness. The examination of the data was conducted following an integrated multi-disciplinary and multi-experimental approach. Emphasis is given on several major aspects of metabolic adaptation to space flight: fluid-electrolyte regulation, mechanisms of hormone disturbances, energy balance and etiology of weight loss. The aim is to obtain a composite picture of the fluid, electrolyte and energy response to weightlessness.

BONE AND CALCIUM

Arnaud CD. Role of dietary calcium in osteoporosis. *Adv Intern Med* 1990;35:93-106. Osteoporosis largely affects white women above the age of 65 years. This, coupled with the fact that life expectancy of this population is now approaching an average of nine decades in the United States, implies that the clinical consequences of osteoporosis will afflict a large and increasing percentage of the internist's patients. In fact, approximately 40% of women will have suffered from wrist or vertebral fractures secondary to osteoporosis after they reach age 65. In his extensive review of this important problem, Dr. Claude Arnaud addresses the critical question of the role of calcium intake both in preventing and treating osteoporosis. This is a controversial issue which requires the very balanced discussion that Dr. Arnaud has provided for the epidemiologic and clinical studies that have addressed this question. There are now good data showing a relationship between low calcium intake in various countries and the incidence of osteoporotic fractures. The data on calcium supplementation, albeit primarily in nonrandomized studies, however, show an inconsistent effect on bone density. Finally, the only study examining the effect of added dietary calcium on fracture prevalence concluded that calcium supplementation reduced the number of fractures by one half. While estrogen replacement remains the most effective means of preventing osteoporosis, Dr. Arnaud concludes that while our knowledge in this field is still rudimentary, for many women calcium supplementation represents a reasonable and safe means of attempting to prevent osteoporosis.

Arnaud CD, and Kolb FO. The calciotropic hormones & metabolic bone disease. In: BASIC AND CLINICAL ENDOCRINOLOGY (Ed.) Greenspan FS. East Norwalk: Appleton & Lange, 1991;247-322.

Arnaud CD, Tsao HS, Littledike T, Hess J, Laakso K, and Bischoff J. Radioimmunoassay of human parathyroid hormone in serum. *J Clin Invest* 1971;50:21-34. A new radioimmunoassay for human parathyroid hormone (PTH) in serum, which can measure the hormone present in 94% of the normal sera tested, is described. It is based on the ability of human PTH to compete with ¹³¹I-labeled bovine PTH for binding to an antiserum directed against porcine PTH. This antiserum distinguishes between human PTH extracted from parathyroid adenomata and that present in hyperparathyroid sera. Evidence is given to suggest that this is due to immunochemical changes in the hormone extracted from adenomata and not to immunochemical heterogeneity of the hormone present in serum.

Physiologic data supporting the validity and specificity of the assay are presented. Induced episodes of hypercalcemia and hypocalcemia resulted in appropriate responses in serum immunoreactive PTH (IPTH) in normal subjects and in patients with Paget's disease of bone. In normals, there was a progressive increase in serum IPTH in the late afternoon and evening, suggesting a diurnal secretory rhythm. A negative correlation was found between the serum calcium and serum IPTH over the normal range of serum calcium values; a positive correlation was found between these variables in patients with primary hyperparathyroidism. There was apparent overlap between serum IPTH values in normal subjects and patients with primary hyperparathyroidism, but formal discriminate analysis of values for serum calcium and IPTH demonstrated separation of these two groups, without overlap.

Bikle DD, Halloran BP, Cone CM, Globus RK, Morey-Holton E. The effects of simulated weightlessness on bone maturation. *Endocrinology* 1987;120(2):678-684. In earlier studies we showed that elevating the hind limbs of growing rats for up to 2 weeks results in a temporary cessation of bone growth in the hind limbs and a transient fall in the serum levels of 1,25-dihydroxyvitamin D. To determine whether such skeletal unloading also retards the maturation of bone, as seen in vitamin D-deprived animals, we fractionated by density the tibiae from rats whose hind limbs had been elevated for up to 15 days. These fractions were analyzed for dry weight, calcium content, and calcium and proline uptake. The most dense fraction (fraction 4) had the highest degree of mineralization (ratio of calcium to dry weight) and comprised

82% of the total dry weight of the control tibiae. The total incorporation of [^3H]proline administered *in vivo* 24 h before removing the tibiae was evenly distributed among all of the fractions, although it was highest in the least dense fraction (fraction 1) when normalized to dry weight. Total incorporation of ^{45}Ca was highest in fraction 4, although when normalized to dry weight it was highest in fraction 3. With skeletal unloading, the portions of bone and ^{45}Ca incorporation in fraction 4 decreased, while the proportions in less dense fractions increased. [^3H]Proline incorporation fell in all fractions. These effects were maximal after 10 days of unloading and returned toward the control values after that time. We conclude that skeletal unloading transiently reduced bone formation and retarded mineralization in the growing rat, which resulted in a decrease in mature bone.

Cann CE. Quantitative CT for determination of bone mineral density: a review. *Radiology* 1988;166(2):509-522. One of the major uses of quantitative computed tomography (CT) has been the measurement of bone mineral density (BMD) at various skeletal sites. The published literature on this subject from 1974 to the present is extensive. Because many investigators and clinicians are just now starting to explore the utility of this technique, the author reviewed this literature to provide both the historic perspective and current status of BMD measurement with CT. The physical and physiologic bases of the method, accuracy, reproducibility, radiation dose, and clinical utility are all discussed.

Cann CE, Henzl M, Burry K, Andreyko J, Hanson F, Adamson GD, Trobough G, Henrichs L, and Stewart G. Reversible bone loss is produced by the GnRH agonist Nafarelin. In: CALCIUM REGULATION AND BONE METABOLISM: BASIC AND CLINICAL ASPECTS, Vol. 9, (Eds.) Cohn DV, Martin TJ, and Meunier PJ. New York: Elsevier Science Publishers B.V., 1987;9:123-127.

Cavanaugh DJ, and Cann CE. Brisk walking does not stop bone loss in postmenopausal women. *Bone* 1988;9:201-204. The rate of loss of spinal trabecular mineral density (TMD) in postmenopausal women, 49-64 years, was measured during a 52 week walking program. The 8 women who walked were 5.6 ± 4.4 years past menopause (mean \pm SD) compared to 6.5 ± 5.1 for 9 nonwalkers. Walkers participated in a progressive walking program for 15-40 min at a heart rate of between 60-85% of maximal age adjusted heart rate, 3 days per week for 52 weeks. Spinal trabecular mineral density was measured using quantitative computed tomography at entry, 6 and 12 months. Pre-exercise heart rate in the walkers decreased 7.8 ± 1.7 beats per min (mean \pm SEM) ($p < 0.01$) from week 0 to week 52, while post-exercise heart rate did not change. Initial spinal mineral density in the walkers was $114 \pm 18 \text{ mg/cm}^3$ (mean \pm SD) and $98 \pm 19 \text{ mg/cm}^3$ in the controls (NS). Bone loss was $5.6 \pm 1.4\%$ (mean \pm SEM) in the walkers and $4.0 \pm 1.2\%$ in the controls; both of these losses were significantly different from zero ($p < 0.005$, $p < 0.01$, respectively), but they were not different from each other. Our study shows that a moderate brisk walking program of one year duration does not prevent the loss of spinal bone density in early-postmenopausal women.

Doty SB. Cell-to-cell communication in bone tissue. In: THE BIOLOGICAL MECHANISMS OF TOOTH ERUPTION AND ROOT RESTORATION (Ed.) Davidovitch Z. Birmingham: EBSCO Media, 1988;61-69. Communication between bone cells is necessary in order to synthesize bone matrix in an organized and structurally responsive manner. Networks of cell-cell communication are morphologically described which include gap junctions and cytoskeletal networks between bone cells, as well as nerve fiber endings in the periosteum which make contact with the periosteal cells. These periosteal cells are coupled, in turn, with adjacent bone cells through gap junctional complexes.

Doty SB, Morey-Holton E, Durnova GN, and Kaplansky AS. Cosmos 1887: morphology, histochemistry, and vasculature of the growing rat tibia. *FASEB J* 1990;4:16-23. Light microscopy, electron microscopy, and enzyme histochemistry were used to study the effects of spaceflight on metaphyseal and cortical bone of the rat tibia. Cortical cross-sectional area and perimeter were not altered by a 12.5-day spaceflight in 3-month-old male rats. The endosteal osteoblast population and the vasculature near the periosteal surface in flight rats compared with ground controls showed more pronounced changes in cortical bone than in metaphyseal bone. The osteoblasts demonstrated greater numbers of transitional Golgi vesicles, possibly caused by a decreased cellular metabolic energy source, but no difference in the large Golgi saccules or the cell membrane-associated alkaline phosphatase activity. The periosteal vasculature in the diaphysis of flight rats often showed lipid accumulations within the lumens of the vessels, occasional degeneration of the vascular wall, and degeneration of osteocytes adjacent to vessels containing intraluminal deposits. These changes were not found in the metaphyseal region of flight animals. The focal vascular changes may be due to ischemia of bone or a developing fragility of the vessel walls as a result of spaceflight.

Fielder PJ, Morey ER, and Roberts WE. Osteoblast histogenesis in periodontal ligament and tibial metaphysis during simulated weightlessness. *Aviat Space Environ Med* 1986;57(12):1125-1130. According to nuclear size, fibroblast-like cells adjacent to bone surfaces in the periodontal ligament (PDL) and tibial primary spongiosa (PS) were classified as less differentiated progenitors and committed osteoprogenitors (A/A'), nonosteogenic cells (B), or preosteoblasts (C/D). The ratio of A/A' to C/D cells reflects osteogenic status of bone lining tissue. When 83-day-old rats were subjected to simulated weightlessness (S-W) for 17 d and examined for changes in osteoblast histogenesis, PDL and PS cell populations increased in A/A' cells ($p < 0.01$; < 0.05) but decreased in C/D cells ($p < 0.01$; < 0.05) compared to controls. These data indicate that the nuclear volume method, originally developed in PDL, can also be used to assess osteoblast histogenesis in PS of long bones, and that simulated weightlessness in the present experimental context interferes with osteoblast histogenesis. Since the surfaces of both weightbearing (PS) and nonweightbearing (PDL) bones were affected, systemic factors appear important in the gravity-related mechanism of osteoblast histogenesis. Although unloading of the tibia and cephalad fluid shifts occur during S-W, the data attained in this experiment could also be explained by stress and/or cessation of growth in the S-W rats.

Garetto LP, Gonsalves MR, Morey ER, Durnova G, and Roberts WE. Preosteoblast production 55 hours after a 12.5-day spaceflight on Cosmos 1887. *FASEB J* 1987;4:24-28. The influence of 12.5 days of spaceflight and a 55 h stressful recovery period (at 1 g) on fibroblastlike osteoblast precursor cells was assessed in the periodontal ligament (PDL) of rats that were 91 days old at launch. Nuclear morphometry was used as a marker for precursor cell differentiation in 3 μm sections cut in the midsagittal plane from the maxillary first molar. According to nuclear volume, cells were classified as preosteoblasts (C + D cells, $\geq 120 \mu\text{m}^3$) and less differentiated progenitor cells (A + A' cells, 40-79 μm^3). Compared with synchronous controls (simulated flight conditions), the 55 h postflight recovery period at 1 g resulted in a 40% decrease in the A + A' cell population, a 42% increase in the C + D cells, and a 39% increase in the number of PDL fibroblastlike cells near the bone surface. These results are consistent with a postflight osteogenic response in PDL. This recovery response occurred despite physiological stress in the flight animals that resulted in a highly significant ($P \leq 0.001$) increase in adrenal weight. The data suggest that after spaceflight there is a strong and rapid recovery mechanism for osteoblast differentiation that is not suppressed by physiological stress.

Globus RK, Bikle DD, Halloran B, and Morey-Holton ER. Skeletal response to dietary calcium in a rat model simulating weightlessness. *J Bone Miner Res* 1986;1(2):191-197. Unweighting the hindlimbs of a rat by tail suspension leads to a decrease in bone in the unweighted hindlimbs, but not in the normally weighted forelimbs. We evaluated whether increments in dietary calcium could prevent this. Growing rats were fed diets ranging in calcium content from 0.1% to 2.4%. After the rats were suspended for two weeks, we found no differences between suspended and control animals fed the same diet with respect to calcium transport or serum levels of calcium, phosphorus, 1,25-dihydroxyvitamin D, and parathyroid hormone. In both groups, increasing dietary calcium reduced active intestinal calcium transport and serum 1,25-dihydroxyvitamin D levels. The calcium content of the tibia and lumbar vertebra (but not the humerus) was reduced in suspended rats compared to control rats fed the same diet. However, increasing dietary calcium increased the calcium content of all bones in both suspended and control animals. The bone formation rate at the tibiofibular junction (measured by double-label tetracycline) was reduced in the suspended animals compared to controls and was not altered by dietary calcium. However, the marrow area of the tibia, an indication of bone resorption, did not differ between suspended and control animals and was equally reduced in both groups when dietary calcium was increased. Our data suggest that the deleterious effects of skeletal unweighting on bone formation cannot be explained by changes in the calciotropic hormones and are not reversed by increments in dietary calcium. However, increasing dietary calcium can increase bone calcium, even in unweighted limbs, by decreasing bone resorption.

Globus RK, Bikle DD, and Morey-Holton E. The temporal response of bone to unloading. *Endocrinology* 1986;118(2):733-742. A model of weightlessness in which the hindlimbs of rats are elevated by their tails at a 40° angle to unload the hindlimbs while maintaining normal weight bearing on the forelimbs has been used to simulate certain conditions of spaceflight. When we used this model in growing rats, we found that growth in bone weight ceased by 1 week in the hindlimbs and lumbar vertebrae, whereas growth in bone weight in the forelimbs and cervical vertebrae remained unaffected. Within 2 weeks, however, the accretion of bone weight in the hindlimbs and lumbar vertebrae returned to normal despite continued skeletal unloading.

Since bone weight in the growing rat is primarily determined by bone formation (bone resorption is modest), we investigated the effects of selective skeletal unloading on bone formation during 2 weeks of hindlimb elevation using radioisotope incorporation (with ⁴⁵Ca and [³H]proline) and histomorphometry (with tetracycline labeling). The studies using radioisotope incorporation showed that bone formation was inhibited by the fifth day of skeletal unloading. By the 10th to 12th day, bone formation had returned toward normal. In comparison with cortical bone, cancellous bone (lumbar vertebrae and proximal tibiae) incorporated more ⁴⁵Ca and [³H]proline (indicating greater metabolic activity) and had a greater absolute response to skeletal unloading. The results of these studies were confirmed by histomorphometric measurements of bone formation using triple tetracycline labeling.

We conclude that this model of simulated weightlessness results in an initial inhibition of bone formation in the unloaded bones. This temporary cessation of bone formation is followed by a cessation in the accretion of bone weight, which then resumes at a normal rate by 14 days despite continued skeletal unloading. We believe that this cycle of inhibition and resumption of bone formation has profound implications for understanding bone dynamics during space flight, immobilization, or bed rest and offers an opportunity to study the hormonal and mechanical factors that regulate bone formation.

Halloran BP, Bikle DD, Wronski TJ, Globus RK, Levens MJ, and Morey-Holton E. The role of 1,25-dihydroxyvitamin D in the inhibition of bone formation induced by skeletal unloading. *Endocrinology* 1986;118(3):948-954. Skeletal unloading results in osteopenia. To examine the involvement of vitamin D in this process, the rear limbs of growing rats were unloaded, and alterations in bone calcium and bone

histology were related to changes in serum calcium (Ca), inorganic phosphorus, 25-hydroxyvitamin D, 24,25-dihydroxyvitamin D [24,25-(OH)₂D], and 1,25-dihydroxyvitamin D [1,25-(OH)₂D]. Acute skeletal unloading induced a transitory inhibition of Ca accumulation in unloaded bones. This was accompanied by a transitory rise in serum Ca, a 21% decrease in longitudinal bone growth ($P < 0.01$), a 32% decrease in bone surface lined with osteoblasts ($P < 0.05$), no change in bone surface lined with osteoclasts, and a decrease in circulating 1,25-(OH)₂D from 130 ± 10 to 53 ± 11 pg/ml. No significant changes in the serum concentrations of inorganic phosphorus, 25-hydroxyvitamin D, or 24,25-(OH)₂D were observed. After 2 weeks of unloading, bone Ca stabilized at approximately 70% of control values, and serum Ca and 1,25-(OH)₂D returned to control values. Maintenance of a constant serum 1,25-(OH)₂D concentration by chronic infusion of 1,25-(OH)₂D (Alza osmotic minipump) throughout the study period did not prevent the bone changes induced by acute unloading. These results suggest that acute skeletal unloading in the growing rat produces a transitory inhibition of bone formation, which, in turn, produces a transitory hypercalcemia, leading to a temporary decrease in serum 1,25-(OH)₂D. No evidence could be found for a direct involvement of 1,25-(OH)₂D in the bone changes induced by skeletal unloading.

Jee WSS, Wronski TJ, Morey ER, and Kimmel DB. Effects of spaceflight on trabecular bone in rats. *Am J Physiol* 1983;244:R310-R314. Alterations in trabecular bone were observed in growing male Wistar rats after 18.5 days of orbital flight on the COSMOS 1129 biosatellite. Spaceflight induced a decreased mass of mineralized tissue and an increased fat content of the bone marrow in the proximal tibial and humeral metaphyses. The osteoblast population appeared to decline immediately adjacent to the growth cartilage-metaphyseal junction, but osteoclast numbers were unchanged. These results suggested that bone formation may have been inhibited during spaceflight, but resorption remained constant. With the exception of trabecular bone mass in the proximal tibia, the observed skeletal changes returned to normal during a 29-day postflight period.

Morey-Holton ER, and Cone CM. Bone as a model system to organ/tissue responses to microgravity. In: FUNDAMENTALS OF SPACE BIOLOGY (Eds.) Asashima M, and Malacinski GM. Tokyo: Japan Science Society Press, 1990;113-122.

Morey-Holton ER, Schnoes HK, DeLuca HF, Phelps ME, Klein RF, Nissenson RH, and Arnaud CD. Vitamin D metabolites and bioactive parathyroid hormone levels during Spacelab 2. *Aviat Space Environ Med* 1988;59(11):1038-1041. The purpose of this study was to determine whether plasma levels of the vitamin D hormone and parathyroid hormone (PTH), two potent activators of bone remodeling sites, were altered in four astronauts during the 8-day (d) Spacelab 2 mission (SL2). Increased circulating levels of either hormone could change calcium homeostasis and bone cell activity and, thus contribute to bone loss in crewmembers in space. The vitamin D hormone was elevated in all astronauts at the end of the first inflight day but returned to normal by the seventh day. Biologically active PTH tended to be normal throughout the mission. Both hormones were within the normal range by the end of the 8-d flight of this SL2 crew. Plasma levels of 25OHD, 24,25(OH)₂D, calcium, phosphorus, and albumin were essentially normal during the mission.

Nissenson RA, Karpf D, Bambino T, Winer J, Canga M, Nyiredy K, and Arnaud CD. Covalent labeling of a high-affinity, guanyl nucleotide sensitive parathyroid hormone receptor in canine renal cortex. *Biochem* 1987;26(7):1874-1878. Putative parathyroid hormone (PTH) receptors in canine renal membranes were affinity labeled with ^{125}I -bPTH(1-34) using the heterobifunctional cross-linking reagent *N*-hydroxysuccinimidyl 4-azidobenzoate. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed the presence of a major 85 000 molecular weight (M_r) PTH binding component, the labeling of which was inhibited by nanomolar concentrations of unlabeled PTH and by micromolar concentrations of 5'-guanylyl imidodiphosphate [Gpp-(NH)p]. Labeling was not influenced by the unrelated peptides insulin and arginine vasopressin. Minor PTH binding components of M_r 55 000 and 130 000 were also seen, and labeling of these was likewise sensitive to unlabeled PTH and to Gpp(GH)p. Omission of protease inhibitors during the isolation of plasma membranes resulted in the loss of the M_r 85 000 PTH binding species and the appearance of an M_r 70 000 form. Several minor PTH binding components also were observed. Equilibrium binding studies showed that such membranes had an affinity for PTH indistinguishable from that in membranes isolated with protease inhibitors and displaying a major M_r 85 000 PTH binding species. We conclude that the major form of the adenylate cyclase coupled PTH receptor in canine renal membranes is an M_r 85 000 protein. An endogenous enzyme, probably a lysosomal cathepsin, can cleave this form to produce an M_r 70 000 receptor that retains full functional activity with respect to high-affinity, guanyl nucleotide sensitive PTH binding. The ability to covalently label the PTH receptor in high yield represents a major step toward the structural characterization of this important detector molecule.

Patterson-Buckendahl P, Arnaud SB, Mechanic GL, Martin RB, Grindeland RE, and Cann CE. Fragility and composition of growing rat bone after one week in spaceflight. *Am J Physiol* 1987;252:R240-R246. To gain some insight into the early effects of spaceflight on skeletal metabolism, we quantified the major chemical constituents and a noncollagenous protein, osteocalcin, in the third-lumbar vertebrae and humeri from 8-wk-old rats that were part of the 7-day NASA Spacelab 3 flight experiments. The ratio of calcium to hydroxyproline in the humeral diaphysis increased from 8.5 in preflight to 9.8 in ground simulation control and only to 8.9 in flight bones. There was no demonstrable change in the fraction of nonmineralized collagen. Osteocalcin content was reduced in the humerus and vertebra. Reduced accumulation of mineral and osteocalcin with no associated decrease in collagen in flight animals suggests that both mineralization and collagen metabolism are impaired in growing animals during spaceflight within a few days after launch. Strength tests of the humeri of flight rats showed substantial deficits that appeared to be related, not only to the reduced bone mass, but also to the composition and quality of new bone formed.

Patterson-Buckendahl P, Globus RK, Bikle DD, Cann CE, and Morey-Holton E. Effects of simulated weightlessness on rat osteocalcin and bone calcium. *Am J Physiol* 1989;257:R1103-R1109. Some of the musculoskeletal changes that occur in growing rats during spaceflight are simulated by a model that selectively unloads the hindlimbs while maintaining normal weight bearing on the forelimbs. Using this model we studied the response of mineral and the mineral-binding protein osteocalcin (OC) in the third lumbar vertebra (L_3) and the femoral midshaft to periods of unweighting from 2 to 28 days. Serum OC decreased by 25%, consistent with a decreased rate of bone growth, during the first week of suspension and returned toward control values after 15 days. The L_3 and femur weighed 20% less than control bones after 10-28 days. OC content of L_3 and femur diaphysis were lower after 7 days of suspension and returned to normal levels at 28 days, whereas Ca content rose slightly at 5 days then decreased sharply. OC:Ca ratio was also affected. The data suggest that unweighting affects formation and deposition of OC and Ca differently depending on bone location and duration of unweighting. Both serum and bone OC are highly sensitive indicators of disruption of osteoblast activity by altered skeletal loading.

Roberts WE, and Morey ER. Proliferation and differentiation sequence of osteoblast histogenesis under physiological conditions in rat periodontal ligament. *Am J Anat* 1985;174:105-118. To define the mechanism of osteoblast histogenesis, nuclear morphometry was utilized as a marker for precursor cell differentiation. One hour after ^3H -thymidine injection, groups of 7-week old rats were killed at hourly intervals over one complete 24-hr photoperiod (LD 12:12). S-phase and mitosis were assessed in autoradiographs of 3- μm sections of molar periodontal ligament (PDL) adjacent to a physiological bone-forming surface. Labeled nuclei were divided into four categories according to morphometry of nuclear sizes: **A** (40-79 μm^3), **B** (80-119 μm^3), **C** (120-169 μm^3), and **D** ($\geq 170 \mu\text{m}^3$) cells. **C** and **D** cells synthesize DNA during the light and divide in the following dark phase; the rhythm for **A** cells is the opposite. **B** cells demonstrated no preference and were subsequently determined to be nonosteogenic. Compared to **A** cells the S-phase photoperiod of **C** and **D** cells (combined) is approximately a one-to-one reciprocal relationship, suggesting two proliferating progenitors in series. Based on arrest points in the histogenesis sequence, five compartments are defined: 1) **A'** cells, less differentiated, self-perpetuating precursors; 2) **A** cells, committed osteoprogenitors; 3) **C** cells, G_1 stage preosteoblasts; 4) **D** cells, G_2 stage preosteoblasts; 5) **Ob** cells, morphologically distinct osteoblasts. Minimal elapsed time for the **A** \rightarrow **A'** \rightarrow **C** \rightarrow **D** \rightarrow **Ob** sequence is about 60 hr (five alternating dark/light cycles). A stress/strain-mediated increase in nuclear volume (**A'** \rightarrow **C**) is an important, rate-limiting step in osteoblast differentiation.

Roberts WE, Fielder PJ, Rosenoer LML, Maese AC, Gonsalves MR, and Morey ER. Nuclear morphometric analysis of osteoblast precursor cells in periodontal ligament, SL-3 rats. *Am J Physiol* 1987;252:R247-R251. Five small (55 days old, 196 ± 5 g)(mean \pm SE) and five large (83 days old, 382 ± 4 g) Sprague-Dawley strain, specific pathogen-free rats were exposed to a 7-day spaceflight and 12-h postflight recovery period. As measured in 3- μm sections, periodontal ligament (PDL) fibroblastlike cells were classified according to nuclear size: **A** + **A'** (40-79), **B** (80-119), **C** (120-169), and **D** ($\geq 170 \mu\text{m}^3$). Since the histogenesis sequence is **A** \rightarrow **A'** \Rightarrow **C** \rightarrow **D** \rightarrow osteoblast, the relative incidence of **A** + **A'** to **C** + **D** is an osteogenic index. No difference in **A** + **A'** or **C** + **D** cells in small rats may reflect partial recovery of preosteoblast formation (**A** \Rightarrow **C**) during the 12-h postflight period. Large flight rats demonstrated increased numbers of **A** + **A'**, indicating an inhibition of preosteoblast formation (**A** \Rightarrow **C**). At least in the older group, a 7-day flight is adequate to reduce PDL osteogenic potential (inhibition in PDL osteoblast differentiation and/or specific attrition of **C** + **D** cells) that does not recover by 12-h postflight.

Sessions NDV, Halloran BP, Bikle DD, Wronski TJ, Cone CM, and Morey-Holton E. Bone response to normal weight bearing after a period of skeletal unloading. *Am J Physiol* 1989;257:E606-E610. Skeletal unloading in the growing rat induces a temporary inhibition of bone formation and thereby a deficit in bone calcium compared with age-matched, normally loaded animals. To determine whether this deficit can be restored by skeletal reloading we measured bone formation rate at the tibiofibular junction and total bone calcium in the tibia and lumbar vertebra in rats whose hindlimbs were unloaded for 2 wk and then reloaded by return to normal weight bearing. Continuously loaded or unloaded animals were also studied. Skeletal unloading reduced bone formation by 34% and tibial and vertebral calcium by 12 and 22%, respectively. Reloading significantly increased the rates of bone formation and calcium accretion 30-34% above normally loaded animals, and by 2 wk had decreased the deficit in tibial and vertebral calcium by 36 and 23%, respectively. These data indicate that the deficit in bone calcium induced by skeletal unloading in the growing rat can be restored in part by return to normal weight bearing. However, the time required to restore bone calcium exceeds the time required to produce the original calcium deficit.

Shaw SR, Vailas AC, Grindeland RE, and Zernicke RF. Effects of a 1-wk spaceflight on morphological and mechanical properties of growing bone. *Am J Physiol* 1988;254:R78-R83. The morphological and mechanical responses of tibia and humerus were assessed in growing male rats after a 1-wk spaceflight aboard NASA Spacelab 3. In contrast to flights of longer duration, changes in middiaphysal cross-sectional morphology were minimal. Inhibition of longitudinal growth was not found in the tibia but was apparent in the humerus. The normal age-related increase in tibial middiaphysal density was not observed in the flight animals. Three-point bending tests indicated that a 1-wk spaceflight impeded the maturation of bone strength and stiffness, with the effects more pronounced in the tibia than in the humerus. Material property alterations in bone thus overshadowed morphological factors in determining the bone's mechanical response. It is likely that deprivation of normal weight-bearing loads was a major factor contributing to the observed changes, but endocrine and other local factors must also be considered.

Shaw SR, Zernicke RF, Vailas AC, DeLuna D, Thomason DB, and Baldwin KM. Mechanical, morphological and biochemical adaptations of bone and muscle to hindlimb suspension and exercise. *J Biomechan* 1987;20(3):225-234. The influences of weightbearing forces on the structural remodeling, matrix biochemistry, and mechanical characteristics of the rat tibia and femur and surrounding musculature were examined by means of a hindlimb suspension protocol and highly intensive treadmill running. Female, young adult, Sprague-Dawley rats were designated as either normal control, sedentary suspended, or exercise suspended rats. For 4 weeks, sedentary suspended rats were deprived of hindlimb-to-ground contact forces, while the exercise suspended rats experienced hindlimb ground reaction forces only during daily intensive treadmill training sessions. The suspension produced generalized atrophy of hindlimb skeletal muscles, with greater atrophy occurring in predominantly slow-twitch extensors and adductors, as compared with the mixed fiber-type extensors and flexors. Region-specific cortical thinning and endosteal resorption in tibial and femoral diaphyses occurred in conjunction with decrements in bone mechanical properties. Tibial and femoral regional remodeling was related to both the absence of cyclic bending strains due to normal weightbearing forces and the decrease in forces applied to bone by antigravity muscles. To a moderate extent, the superimposed strenuous running counteracted muscular atrophy during the suspension, particularly in the predominantly slow-twitch extensor and adductor muscles. The exercise did not, however, mitigate changes in bone mechanical properties and cross-sectional morphologies, and in some cases exacerbated the changes. Suspension with or without exercise did not alter the normal concentrations of collagen, phosphorus, and calcium in either tibial or femur.

Turner RT, Bell NH, Duvall P, Bobyn JD, Spector M, Morey-Holton E, and Baylink DJ. Spaceflight results in formation of defective bone. *Proc Soc Exp Biol Med* 1985;180:544-549. Growing rats were flown on 19 day spaceflights aboard Cosmos 782 and 936 biosatellites. Spaceflight resulted in a prominent skeletal defect of the periosteal surface of the tibia diaphysis. The defect, termed an arrest line, was approximately 3 μ m across and separated the bone formed in space from that formed following spaceflight. The bone matrix at the arrest line region was abnormal in that collagen fibers were preferentially orientated parallel to the periosteal surface. In addition, the bone matrix was hypomineralized. The altered bone was inferior to normal bone in resistance to abrasion and may be partially responsible for the decrease in torsional strength observed after spaceflight.

Vailas AC, Deluna DM, Lewis LL, Curwin SL, Roy RR, and Alford EK. Adaptation of bone and tendon to prolonged hindlimb suspension in rats. *J Appl Physiol* 1988;65(1):373-376. The rat hindlimb suspension model was used to ascertain the importance of ground reaction forces in maintaining bone and tendon homeostasis. Young female Sprague-Dawley rats were randomly assigned to either a suspended or a nonsuspended group. After 28 days, femur bones and patellar tendons were obtained for morphological and biochemical analyses. Prolonged suspension induced a significant change in the geometric configuration of the femur middiaphysis by increasing the minimum diameter (12%) without any significant alterations in cortical area, density, mineral, and collagen concentrations. Femur wet weight, length, DNA, and uronic acid concentrations of suspended animals were not significantly different from bones of nonsuspended rats. However, the collagen and proteoglycan concentrations in patellar tendons of suspended rats were 28% lower than the concentrations of matrix proteins in tissues obtained from nonsuspended animals. These data suggest that elimination of ground reaction forces induces alterations in tendon composition and femur diaphyseal shape by changing regional rates in bone remodeling and localized tendon strain. Therefore it appears that ground reaction forces are an important factor in the maintenance of cortical bone and patellar tendon homeostasis during weight-bearing conditions.

Vailas AC, Zernicke RF, Grindeland RE, Kaplansky A, Durnova GN, Li KC, and Martinez DA. Effects of spaceflight on rat humerus geometry, biomechanics, and biochemistry. *FASEB J* 1990;4:47-54. The effects of a 12.5 day spaceflight (Cosmos 1887 biosatellite) on the geometric, biomechanical, and biochemical characteristics of humeri of male specific pathogen-free rats were examined. Humeri of age-matched basal control, synchronous control, and vivarium control rats were contrasted with the flight bones to examine the influence of growth and space environment on bone development. Lack of humerus longitudinal growth occurred during the 12.5 days in spaceflight. In addition, the normal mid-diaphysial periosteal appositional growth was affected; compared with their controls, the spaceflight humeri had less cortical cross-sectional area, smaller periosteal circumferences, smaller anterior-posterior periosteal diameters, and smaller second moments of area with respect to the bending and nonbending axes. The flexural rigidity of the flight humeri was comparable to that of the younger basal control rats and significantly less than that of the synchronous and vivarium controls; the elastic moduli of all four groups, nonetheless, were not significantly different. Generally, the matrix biochemistry of the mid-diaphysial cross sections showed no differences among groups. Thus, the spaceflight differences in humeral mechanical strength and flexural rigidity were probably a result of the differences in humeral geometry rather than material properties.

Vailas AC, Zernicke RF, Matsuda J, Curwin S, and Durivage J. Adaptation of rat knee meniscus to prolonged exercise. *J Appl Physiol* 1986;60(3):1031-1034. The morphological and biochemical adaptations of knee meniscus to prolonged exercise were studied. Female Sprague-Dawley rats maintained under controlled environmental conditions were randomly assigned to either an endurance-trained or a sedentary group. Training consisted of a progressive protocol on a motor-driven treadmill, 5 days/wk for 12 wk. Knee lateral menisci were obtained from anesthetized rats and used for morphological and biochemical analyses. Gastrocnemius succinate dehydrogenase increased 65% in the endurance-trained group, as evidence for a training effect. In the trained group, collagen, proteoglycan, and calcium concentrations increased significantly in the posterior region of the lateral meniscus. In contrast, no significant changes were found in the anterior region of the lateral meniscus. The region-specific changes in meniscal concentrations of calcium and matrix macromolecules in response to prolonged exercise are consistent with the distinctly different mechanical properties and functional roles of the anterior and posterior regions of the rat knee meniscus.

Wronski TJ, and Morey ER. Effect of spaceflight on periosteal bone formation in rats. *Am J Physiol* 1983;244:R305-R309. Male Wistar rats were placed in orbit for 18.5 days aboard the Soviet COSMOS 1129 biological satellite. Tetracycline was administered before and after spaceflight to label areas of bone formation. An inhibition of periosteal bone formation occurred during spaceflight in the tibial and humeral diaphyses, but this defect was corrected during the postflight period. The increased extent of arrest lines at these skeletal sites suggested that periosteal bone formation may have even ceased during spaceflight. The rib exhibited a small but nonsignificant decrease in periosteal bone formation. Endosteal bone resorption was not affected markedly by spaceflight conditions. The observed inhibition of periosteal bone formation may be a result of mechanical unloading, but endocrine factors cannot be ruled out.

Wronski TJ, and Morey-Holton ER. Skeletal response to simulated weightlessness: a comparison of suspension techniques. *Aviat Space Environ Med* 1987;58(1):63-68. The skeletal response to simulated weightlessness was studied in rats subjected to two different methods of suspension. Skeletal unloading of the hind limbs for a two week period was achieved by use of either a back harness or tail traction. In comparison to pair-fed control rats, back-suspended rats failed to gain weight whereas tail-suspended rats exhibited normal weight gain. Quantitative bone histomorphometry revealed marked skeletal abnormalities in the proximal tibial metaphysis of back-suspended rats. Loss of trabecular bone mass in these animals was due to a combination of depressed longitudinal bone growth, decreased bone formation, and increased bone resorption. In contrast, the proximal tibia of tail-suspended rats was relatively normal by these histologic criteria. However, a significant reduction in trabecular bone volume occurred during 2 weeks of tail suspension, possibly due to a transient inhibition of bone formation during the early stages of skeletal unloading. Lack of weight gain in back-suspended rats may be indicative of a pronounced stress response during which corticosteroids adversely affected the skeleton. Maintenance of normal weight gain by tail-suspended rats provides evidence for the less traumatic nature of this method of suspension. Our findings indicate that tail suspension may be a more appropriate model for evaluating the effects of simulated weightlessness on skeletal homeostasis.

Wronski TJ, Morey-Holton ER, Doty SB, Maese AC, and Walsh CC. Histomorphometric analysis of rat skeleton following spaceflight. *Am J Physiol* 1987;252:R252-R255. Male Sprague-Dawley rats were placed in orbit for 7 days aboard the space shuttle. Bone histomorphometry was performed in the long bones and lumbar vertebrae of flight rats and compared with data derived from ground-based control rats. Trabecular bone mass was not altered during the 1st wk of weightlessness. Strong trends were observed in flight rats for decreased periosteal bone formation in the tibial diaphysis, reduced osteoblast size in the proximal tibia, and decreased osteoblast surface and number in the lumbar vertebra. For the most part, histological indexes of bone resorption were normal in flight rats. The results indicate that 7 days of weightlessness are not of sufficient duration to induce histologically detectable loss of trabecular bone in rats. However, cortical and trabecular bone formation appear to be diminished during the 1st wk of spaceflight.

MUSCLE

Curwin SL, Vailas AC, and Wood J. Immature tendon adaptation to strenuous exercise. *J Appl Physiol* 1988;65(5):2297-2301. White Leghorn roosters (3 wk old) were randomly assigned to runner or control groups. Runners were subjected to a progressive treadmill running program for 8 wk, 5 days/wk at 70-80% maximal O_2 consumption (VO_{2max}). After 8 wk, runners showed a significant elevation in gastrocnemius fumarase activity (51%) and a 21 % increase in VO_{2max} compared with controls. The exercise program induced a significant increase in tendon collagen deposition (46%) without any changes in DNA, proteoglycan, and collagen concentrations or tendon dry weight. Also, tendon collagen from runners contained fewer (50%) pyridinoline cross-links. These results suggest that high-intensity exercise causes greater matrix-collagen turnover in growing chickens, resulting in reduced maturation of tendon collagen.

Ellis S, Giometti CS, and Riley DA. Changes in muscle protein composition induced by disuse atrophy: analysis by two-dimensional electrophoresis. *Physiologist* 1985;28(6,Suppl):S159-S160. Two dimensional (2-D) electrophoresis has been used extensively to map the major proteins of rabbit, rat, chicken and human muscle. However, it has been little used for investigating possible changes in the broad spectrum of proteins in muscle which has undergone atrophy due to load removal. By this technique it was shown that the myosin light chains and tropomyosins shift from the typical slow-twitch muscle pattern to a fast-twitch pattern after a long duration inactivity of the hind limb muscles of the cat produced by cord transection. Six week immobilization of rat hindlimbs showed no changes by 2-D electrophoresis in the characteristic myosin light chains in either the soleus or vastus lateralis. One and four weeks of immobilization produced significant alterations in myosin light chains and related peptides in 2-D electrophoresis. However, in addition to the reported changes in contractile proteins, there are indications that there may occur substantial changes in the relative quantities, and perhaps even quality, of non-contractile skeletal muscle proteins following disuse atrophy. This was the rationale for undertaking a 2-D electrophoretic analysis of the broad spectrum of muscle proteins in the soleus and EDL muscles from hindlimbs maintained load-free for 10 days.

Fahlman CS, and Riley DA. Colchicine-induced sprouting of the neuromuscular junction in the pigeon extensor digitorum longus muscle. *Brain Res* 1986;363:156-160. Colchicine-induced motor endplate sprouting in the extensor digitorum longus muscle of the pigeon was examined. Ten days after the drug application sprouting from the endplate arborizations and nodes of Ranvier were observed. No concomitant changes in endplate surface area or in the degree of terminal branching could be demonstrated. Similarities between the sprouting patterns of the pigeon endplate and the mammalian endplate are discussed.

Fitts RH, Metzger JM, Riley DA, and Unsworth BR. Models of disuse: a comparison of hindlimb suspension and immobilization. *J Appl Physiol* 1986;60(6):1946-1953. The effects of 1 and 2 wk of hindlimb suspension (HS) on rat skeletal muscle function were determined and the results compared with those obtained previously with hindlimb immobilization (HI). Both models of disuse (HS and HI) primarily affected slow-twitch muscle. Each decreased the isometric twitch duration in the slow-twitch soleus; however, the HS-mediated effect was entirely a result of a shortened contraction time (CT), whereas HI reduced one-half relaxation time ($1/2$ RT) as well as CT. Soleus muscle mass and peak tetanic tension (P_0) declined with disuse. The HS effect on muscle mass and P_0 was variable, however, for all experiments HS produced atrophy equal to or greater than HI. A major difference existed in the effects of HS and HI on the maximal speed of soleus muscle shortening (V_{max}). One and 2 wk of HS produced increases in V_{max} to 4.45 ± 0.34 and 6.83 ± 0.74 fiber lengths/s, respectively, compared with control velocities of 3.05 ± 0.08 . By contrast over a similar time period, HI had no significant effect on soleus V_{max} . The increase in V_{max} at 14

days of HS was associated with, and perhaps caused by, the increased expression of a second faster migrating isozyme of myosin. The new native isozyme comigrated with fast myosin, but its light chain subunits contained only LC_{1s} and LC_{2s}. The mechanism responsible for the increase is unknown. One plausible explanation is that the apparent HS-mediated modification in muscle fiber type is dependent on the elimination of loadbearing or isometric contractions, a condition that does not exist during HI.

Hoh JFY, Hughes S, Hale PT, and Fitzsimons RB. Immunocytochemical and electrophoretic analyses of changes in myosin gene expression in cat limb fast and slow muscles during postnatal development.

J Muscle Res Cell Motil 1988;9:30-47. Changes in myosin synthesis during the postnatal development of the fast extensor digitorum longus (EDL) and the slow soleus muscles of the kitten were examined using immunocytochemical techniques supplemented by pyrophosphate gel electrophoresis and gel electrophoresis-derived enzyme linked immunosorbent assay (GEDELISA) of myosin isoforms. The antibodies used were monoclonals against heavy chains of slow and fast myosins and a polyclonal against foetal/embryonic myosin. In both muscles in the newborn kitten, there was a population of more mature fibres which stained strongly for slow but weakly for foetal/embryonic myosin. These fibres were considered to be primary fibres. They formed 4.8% of EDL fibres and 26% of soleus fibres at birth, and continued to express slow myosin in adult muscles. The less mature secondary fibres stained strongly for foetal/embryonic myosin, and these could be divided into two subpopulations; fast secondaries in which foetal/embryonic myosin was replaced by fast myosin, and slow secondaries in which the myosin was replaced by slow myosin. At 50 days the EDL had a large population of fast secondaries (83% of total fibres) and a small population of slow secondaries which gradually transformed into fast fibres with maturity. The vast majority of secondary fibres in the soleus were slow secondaries, in which slow myosin synthesis persisted in adult life. There was a restricted zone of fast secondaries in the soleus, and these gradually transformed into slow fibres in adult life. It is proposed that the emergence of primary fibres and the two populations of secondary fibres is myogenically determined.

Riley DA, Bain JLW, Ellis S, and Haas AL. Quantitation and immunocytochemical localization of ubiquitin conjugates within rat red and white skeletal muscles. *J Histochem Cytochem* 1988;36(6):621-632. We employed solid-phase immunochemical methods to probe the dynamics of ubiquitin pools within selected rat skeletal muscles. The total ubiquitin content of red muscles was greater than that of white muscles, even though the fractional conjugation was similar for both types of muscle. The specificity for conjugated ubiquitin in solid-phase applications, previously demonstrated for an affinity-purified antibody against SDS-denatured ubiquitin, was retained when used as a probe for ubiquitin-protein adducts in tissue sections. Immunohistochemical localization revealed that differences in ubiquitin pools derived from the relative content of red (oxidative) vs white (glycolytic) fibers, with the former exhibiting a higher content of ubiquitin conjugates. Subsequent immunogold labeling demonstrated statistically significant enhanced localization of ubiquitin conjugates to the Z-lines in both red and white muscle fiber types.

Riley DA, Ellis S, and Bain JLW. Catalase-positive microperoxisomes in rat soleus and extensor digitorum longus muscle fiber types. *J Histochem Cytochem* 1988;36(6):633-637. The size, distribution, and content of catalase-reactive microperoxisomes were studied cytochemically in slow-twitch oxidative (SO), fast-twitch oxidative glycolytic (FOG), and fast-twitch glycolytic (FG) fibers of soleus and extensor digitorum longus (EDL) rat muscles. Fiber types were classified on the basis of mitochondrial content and distribution, Z-band widths, and myofibril size and shape. Microperoxisomes were generally located between myofibrils at the I-bands. The absence of crystalloid inclusions prevented positive identification of microperoxisomes in nonreacted and aminotriazole-inhibited muscles. EDL and soleus SO fibers

possessed the largest microperoxisomes, whereas FOG and FG fibers of the EDL contained small- to medium-sized microperoxisomes. Comparing either microperoxisome number per muscle fiber area or microperoxisome area per fiber area revealed significant differences between fiber types with this ranking: soleus SO > EDL SO > EDL FOG > EDL FG. The present observations demonstrate that the content of catalase-positive microperoxisomes is greatest in the oxidative muscle fiber types. These cytochemical findings account for the higher catalase activity in homogenates of soleus muscles as compared to that of EDL muscles, because the soleus contains more oxidative fibers than EDL.

Riley DA, Ellis S, Slocum GR, Satyanarayana T, Bain JLW, and Sedlak FR. Hypogravity-induced atrophy of rat soleus and extensor digitorum longus muscles. *Muscle Nerve* 1987;10:560-568. Prolonged exposure of humans to hypogravity causes weakening of their skeletal muscles. This problem was studied in rats exposed to hypogravity for 7 days aboard Spacelab 3. Hindlimb muscles were harvested 12-16 hours postflight for histochemical, biochemical, and ultrastructural analyses. The majority of the soleus and extensor digitorum longus fibers exhibited simple cell shrinkage. However, approximately 1% of the fibers in flight soleus muscles appeared necrotic. Flight muscle fibers showed increased glycogen, lower subsarcolemmal staining for mitochondrial enzymes, and fewer subsarcolemmal mitochondria. During atrophy, myofibrils were eroded by multiple focal losses of myofilaments; lysosomal autophagy was not evident. Tripeptidylaminopeptidase and calcium-activated protease activities of flight soleus fibers were significantly increased, implying a role in myofibril breakdown. Simple fiber atrophy appears to account for muscle weakening during spaceflight, but fiber necrosis is also a contributing factor.

Riley DA, Ellis S, Slocum GR, Satyanarayana T, Bain JLW, and Sedlak FR. Morphological and biochemical changes in soleus and extensor digitorum longus muscles of rats orbited in Spacelab 3. *Physiologist* 1985;28(6,Suppl):S207-S208. Flight rats were exposed to 7 days of hypogravity, and control animals remained on earth in simulated flight cages. Hindlimb muscles were harvested 12-16 hours postflight. Mean soleus fiber area decreased 35.8%. Extensor digitorum longus (EDL) fibers atrophied 24.9%. Most atrophic fibers were small versions of control fibers, indicating simple cell shrinkage. However, up to 1% of the fibers in flight soleus muscles appeared undergoing cell death. Also present were large round fragmented fibers possibly broken during the postflight exposure to terrestrial gravity or during tissue processing. Both the EDL and soleus muscles acquired fast histochemical properties, i.e., increases in alkaline myofibrillar ATPase, alpha glycerophosphate dehydrogenase, glycogen and a decrease in NADH dehydrogenase staining. Myofibrils of flight solei were eroded by multiple focal losses of myofilaments. Following flight, tripeptidylaminopeptidase and total calcium activated protease activities were significantly increased by 60% and 26% respectively. These two proteases possibly function in myofibril breakdown. The muscle fiber changes described herein cannot be attributed solely to hypogravity because of the long postflight exposure of the rats to terrestrial gravity.

Riley DA, and Fahlman CS. Colchicine-induced differential sprouting of the endplates on fast and slow muscle fibers in rat extensor digitorum longus, soleus and tibialis anterior muscles. *Brain Res* 1985;329:83-95. The patterns of sprouting of motor endplates were examined in fast extensor digitorum longus and slow soleus muscles and in tibialis anterior muscles containing fast and slow muscle fiber types. A histochemical technique combining nerve silver impregnation and endplate cholinesterase staining was developed for this task. Temporal examination of the innervation was conducted 3, 7, and 10 days after either a 45 or 90 min application of the ipsilateral sciatic nerve with 5 mM colchicine. This dosage of drug did not cause detectable axon or muscle fiber degeneration, unlike 60 mM which was highly neurotoxic. At 3 days following treatment with the lower concentration, there were no significant differences in the percentages

of intranodal, preterminal and ultraterminal sprouts between the normal (non-treated), sham-treated, contralateral systemic-control and drug-treated groups of muscles. By 7 and 10 days, the muscles on the drug-treated side exhibited significant increases in the 3 types of sprouts. Collateral sprouting was uncommon: most outgrowths remained on the muscle fibers innervated by the parent axons. Endplates in the tibialis anterior muscles of the control and drug-treated groups were classified Complex, Intermediate or Simple according to the relative degrees of branching of the terminal arbors. The occurrence of endplate classes and muscle fiber types was correlated in the superficial and deep regions of this muscle. Complex endplates innervated fast glycolytic fibers, Intermediate endplates supplied fast oxidative glycolytic fibers, and Simple endplates served slow oxidative fibers. In response to colchicine, the endplates of the slow muscles sprouted more than those of fast muscles while the innervation of slow fiber types sprouted less than that of fast fiber types. Furthermore, intranodal sprouts were more prevalent in slow muscles and ultraterminal sprouts more numerous in fast muscles whereas intranodal sprouts predominated on fast fiber types and ultraterminal sprouts were characteristic of slow fiber types. These apparently contradictory results were reconciled when it was noted that soleus endplates were mostly Complex and Intermediate, and the extensor digitorum longus contained more Simple endplates. Thus, consistency of sprouting patterns among endplate types of the 3 muscles was recognized when the pre-existing branching patterns were considered. This indicated that the patterns of sprouting were determined by the motor neurons rather than the muscle fibers. The observed sprouting responses supported the hypothesis that colchicine treatment of motor axons caused muscle fibers to elaborate a diffusible sprout-inducing factor.

Riley DA, Ilyina-Kakueva EI, Ellis S, Bain JLW, Slocum GR, and Sedlak FR. Skeletal muscle fiber, nerve, and blood vessel breakdown in space-flown rats. *FASEB J* 1990;4:84-91. Histochemical and ultrastructural analyses were performed postflight on hind limb skeletal muscles of rats orbited for 12.5 days aboard the unmanned Cosmos 1887 biosatellite and returned to Earth 2 days before sacrifice. The antigravity adductor longus (AL), soleus, and plantaris muscles atrophied more than the non-weight bearing extensor digitorum longus, and slow muscle fibers were more atrophic than fast fibers. Muscle fiber segmental necrosis occurred selectively in the AL and soleus muscles; primarily, macrophages and neutrophils infiltrated and phagocytosed cellular debris. Granule-rich mast cells were diminished in flight AL muscles compared with controls, indicating that mast cell secretion contributed to interstitial tissue edema. Increased ubiquitination of disrupted myofibrils implicated ubiquitin in myofilament degradation. Mitochondrial content and succinic dehydrogenase activity were normal, except for subsarcolemmal decreases. Myofibrillar ATPase activity of flight AL muscle fibers shifted toward the fast type. Absence of capillaries and extravasation of red blood cells indicated failed microcirculation. Muscle fiber regeneration from activated satellite cells was detected. About 17% of the flight AL end plates exhibited total or partial denervation. Thus, skeletal muscle weakness associated with spaceflight can result from muscle fiber atrophy and segmental necrosis, partial motor denervation, and disruption of the microcirculation.

Riley DA, Sanger JR, Matloub HS, Yousif NJ, Bain JLW, and Moore GH. Identifying motor and sensory myelinated axons in rabbit peripheral nerves by histochemical staining for carbonic anhydrase and cholinesterase activities. *Brain Res* 1988;453:79-88. Carbonic anhydrase (CA) and cholinesterase (CE) histochemical staining of rabbit spinal nerve roots and dorsal root ganglia demonstrated that among the reactive myelinated axons, with minor exceptions, sensory axons were CA positive and CE negative whereas motor axons were CA negative and CE positive. The high specificity was achieved by adjusting reaction conditions to stain subpopulations of myelinated axons selectively while leaving 50% or so unstained. Fixation with glutaraldehyde appeared necessary for achieving selectivity. Following sciatic nerve transection, the reciprocal staining pattern persisted in damaged axons and their regenerating processes which formed neuromas within the proximal nerve stump. Within the neuromas, CA-stained

sensory processes were elaborated earlier and in greater numbers than CE-stained regenerating motor processes. The present results indicate that histochemical axon typing can be exploited to reveal heterogeneous responses of motor and sensory axons to injury.

Riley DA, and Slocum GR. Contraction-free, fume-fixed longitudinal sections of fresh frozen muscle. *Stain Technol* 1988;63(2):93-96. Contraction damage occurring when longitudinal frozen sections of fresh unfixed muscles are thawed on microscope slides has limited histological examination of this tissue mainly to cross sections. Longitudinally oriented sections are advantageous for investigating properties that vary along the length of the muscle fibers. A fume fixation technique has been developed for preventing contraction of thick longitudinal frozen sections. The technique is compatible with histochemical staining of enzymes.

Yip RK, and Riley DA. Effects of methylmercury on the motor and sensory innervation of the rat extensor digitorum longus muscle. *Environ Res* 1987;43:85-96. This histochemical study examined the effects of chronic methylmercury (MeHg) intoxication on the motor and sensory innervation of extensor digitorum longus muscles. Light microscopic examination of silver-stained axons in the intramuscular nerve bundles of MeHg-treated rats showed Wallerian-like degeneration and a reduction in the number of nerve fibers. Disrupted axons were predominantly sensory because 22.2% of spindle afferents (I_a) and 90.0% of Golgi tendon organ (I_b) sensory fibers were completely degenerated whereas less than 1% of motor endings were totally destroyed. Partial disruption occurred in the cholinesterase and motor terminals of 13.7% of endplates. Our results demonstrated greater vulnerability of sensory nerves than of motor nerves to MeHg-induced degeneration. Thus, the abnormal reflexes, ataxia, and muscle weakness following MeHg poisoning appear related to reduction of proprioceptive feedback from muscles and tendons in addition to the documented lesions in the central nervous system.

HEMATOLOGY AND IMMUNOLOGY

Bechler B, Cogoli A, and Mesland D. Lymphozyten sind schwerkraftempfindlich (Are lymphocytes sensitive to gravitational forces?). *Naturwissenschaften* 1986;73:400-403. Two experiments dedicated to the study of human lymphocyte activation by the mitogen concanavalin A (Con A) in microgravity were performed. All cultures in micro-g had a corresponding inflight 1-g control in a reference centrifuge. In all cultures kept under micro-g conditions, activation by Con A is depressed by more than 90% as compared to the control. Activation of lymphocytes from the crew members is markedly depressed in the 1-g inflight as well as in the samples drawn 1 h after landing, as compared to the preflight values. Activation recovers to preflight level between 7 and 13 days after landing.

Cogoli A, Bechler B, Müller O, and Hunzinger E. Effect of microgravity on lymphocyte activation. In: **BIORACK ON SPACELAB D1** (Eds.) Logdon N, and David V. Noordwijk: ESA Publications Division (ESA SP-1091), 1988;89-100.

Cogoli A, and Tschopp A. Effect of spaceflight on lymphocyte stimulation. *Physiologist* 1980;23(6,Suppl):S63-S66.

Cogoli A, and Tschopp A. Lymphocyte reactivity during spaceflight. *Immunol Today* 1985;6(1):1-4. Over twenty years of spaceflight have demonstrated that man can easily survive and work in weightless conditions. However, a number of physiological changes may affect crew performance in space. Besides the well known disturbances of the vestibular and cardiovascular systems, bone demineralization, and decrease of erythrocyte mass, certain immunological alterations have been observed in space crews after flight. One of them - the reduction of lymphocyte reactivity to mitogens - was the subject of our investigations during the flight of Spacelab 1 from 28 November - 8 December 1983.

Dunn CRD, Johnson PC, and Lange RD. Regulation of hematopoiesis in rats exposed to antiorthostatic hypokinetic/hypodynamia: II. mechanisms of the "anemia". *Aviat Space Environ Med* 1986;57(1):36-44. Results are presented which demonstrate a close similarity between the ability of hypokinetic/hypodynamia and orthostatic hypokinetic/hypodynamia to induce anemia in laboratory rats. The "restraint anemia" (whether mediated directly by reduced activity or indirectly by possible changes in blood circulation or in altered weight-bearing capacity of the skeleton) was largely due to reduced food and/or water consumption and displayed the classical symptoms of inadequate nutrition, i.e. decreased serum erythropoietin (Ep) titers and reduced Ep sensitivity of hematopoietic tissue. Only changes in red blood cell (RBC) clearance were unique to the head-down (antiorthostatic) posture. During suspension, RBC clearance was reduced and then accelerated when suspension was terminated or the cells transfused into a normal environment. Changes in RBC clearance were due to both cell-associated and cell-independent factors and may be related to the alterations in RBC survival seen in rats during or immediately after space flight. In both suspension and weightlessness, these changes were limited to alterations in the force and/or direction of the gravity vector.

Dunn CDR, Johnson PC, Lange RD, Perez L, and Nessel R. Regulation of hematopoiesis in rats exposed to antiorthostatic, hypokinetic/hypodynamia: I. model description. *Aviat Space Environ Med* 1985;56(5):419-426. This paper provides baseline information regarding the regulation of hematopoiesis in antiorthostatic, hypokinetic/hypodynamic ("suspended") laboratory rats. The object of the study was to compare the hematological effects of suspension with those seen following space flight in man and/or rats.

Observed in man after exposure to microgravity and in the suspended rats was a reduced red blood cell mass, suppressed erythropoiesis, a transient increase in hematocrit due to a reduction in plasma volume, a post-exposure hematocrit decrease, a weight loss (or failure to thrive) and a reduction in food and water consumption. A rightward shift in the oxyhemoglobin dissociation curve, observed in the rat "model", has been predicted to occur during manned space flight but has not yet been measured. Suppression of hematopoiesis is a common feature of rats during both space flight and suspension. Platelet counts showed no significant change in rats after suspension or in man during space flight. Unlike man in space but similar to space flight-exposed rats, no significant change in leukocyte number or reactivity to PHA *in vitro*, or in red blood cell shape distribution were observed in the suspended rats. At least in a gross sense, the rat "model" seems to reproduce many of the known hematological effects of space flight and offers promise as a 1-g analog for understanding hematopoietic effects similar to those found in space flight.

Gmünder FK, Lorenzi G, Bechler B, Joller P, Müller J, Ziegler WH, and Cogoli A. Effect of long-term physical exercise on lymphocyte reactivity: similarity to spaceflight reactions. *Aviat Space Environ Med* 1988;59(2):146-151. The response of critical immunological parameters in seven athletes to the sustained physical stress of marathon running was assessed. Variables analysed were the responsiveness of lymphocytes (measured as mitogenic response to concanavalin A), the numbers of lymphocytes, their subsets, and leukocyte numbers. In addition, blood levels of cortisol, epinephrine, and norepinephrine were determined. After the run, lymphocyte responsiveness was severely depressed to 1-70% of the resting values, even though the lymphocyte counts did not change. Leukocyte counts were elevated 2.8-fold. No dramatic changes were found within the lymphocyte subsets, although an increase in pan T-cells and the helper/inducer subset 2 d after the run was significant. In addition, the numbers of B-cells decreased significantly. No change was observed within the suppressor/cytotoxic subset. Cortisol increased 2.1-fold, epinephrine 3.2-fold, and norepinephrine 2.7-fold. All these parameters returned to baseline values within 2 d. These data were compared with data obtained during and after spaceflight. We conclude that prolonged physical stress of marathon running induces changes in immunological responsiveness that are strikingly similar to those arising from the stress of spaceflight.

Lange RD, Andrews RB, Gibson LA, Congdon CC, Wright P, Dunn CDR, and Jones JB. Hematological measurements in rats flown on Spacelab shuttle, SL-3. *Am J Physiol* 1987;252:R216-R221. Previous studies have shown that a decrease in red cell mass occurs in astronauts, and some studies indicate a leukocytosis occurs. A life science module housing young and mature rats was flown on shuttle mission Spacelab 3 (SL-3), and the results of hematology studies of flight and control rats are presented. Statistically significant increases in the hematocrit, red blood cell counts, and hemoglobin determinations, together with a mild neutrophilia and lymphopenia, were found in flight animals. No significant changes were found in bone marrow and spleen cell differentials or erythropoietin determinations. Clonal assays demonstrated an increased erythroid colony formation of flight animal bone marrow cells at erythropoietin doses of 0.02 and 1.0 U/ml but not 0.20 U/ml. These results agree with some but vary from other previously published studies. Erythropoietin assays and clonal studies were performed for the first time.

Lange RD, Andrews RB, Gibson LA, Wright P, Dunn CDR, and Jones JB. Hematological studies on rats flown on shuttle flight SL-3. In: REGULATION OF ERYTHROPOIESIS (Eds.) Lange ED, Tavassoli M, and Ascensao JL. New York: PMA Publishing Co., 1988;455-466. Astronauts who have flown in microgravity have experienced a loss of red blood cell mass. The pathogenesis of this anemia of space flight has not been ascertained, although it is probably multifactorial. A few experiments have been conducted on laboratory animals which demonstrate some of the same changes found in human astronauts.

From 04/29/85 to 05/06/85, 24 white rats were flown on the SL-3 mission of the shuttle Challenger. Although this was primarily an engineering flight, these animals were studied upon return. The results of hematologic studies are presented in this chapter.

Lange RD, Andrews RB, Gibson LA, Wright P, Dunn CDR, and Jones JB. Hematologic parameters of astrorats flown on SL-3. *Physiologist* 1985;28(6,Suppl):S195-S196. Hematologic studies were performed on a group of large and small rats which were sacrificed after flying in life sciences shuttle engineering flight SL-3. The results are presented on flight (F) and control (C) 200 gm rats.

The small flight animals demonstrated a significant increase in hematocrits, red blood cell counts, hemoglobins and peripheral blood percentages of neutrophils as well as a decrease in percentage of lymphocytes. Erythropoietin (Ep) determinations were similar for the two groups as were the bone marrow and spleen differential counts. *In vitro* cultures for erythroid colonies of bone marrow showed that in response to different doses of Ep, in all cases where differences were statistically significant, the F rats had increased colony counts.

The changes in red cell parameters could be caused by a decrease in plasma volume. However, no isotopic studies were possible on this flight and this lack points up the need for such studies to determine the red cell mass and plasma volume.

Leach CS, Chen JP, Crosby W, Johnson PC, Lange RD, Larkin E, and Tavassoli M. Hematology and biochemical findings of Spacelab 1 flight. In: REGULATION OF ERYTHROPOIESIS (Eds.) Zanjani ED, Tavassoli M, and Ascensao JL. New York: PMA Publishing Corp., 1988;415-453. The most consistent finding in studies of the influence of space flight on the hematologic system of man has been a significant reduction in the circulating red cell mass (RCM). This phenomenon has been observed in the American Gemini, Apollo, Skylab, and Apollo-Soyuz Test Project missions, and Soviet Soyuz-Salyut missions. Data from the Skylab flights suggest that suppression of normal erythropoiesis may be a cause of red cell mass reduction found after space flight.

An experiment conducted on the 10-day Spacelab 1 mission aboard the ninth Space Shuttle flight in November to December 1983 was designed to measure factors involved in the control of erythrocyte turnover - particularly erythropoiesis - in man which might be altered soon after the beginning of exposure to weightlessness. Many of these hematological and biochemical parameters have not previously been measured in blood specimens collected during space flight.

Lorenzi G, Fuchs-Bislin P, and Cogoli A. Effects of hypergravity on "whole-blood" cultures of human lymphocytes. *Aviat Space Environ Med* 1986;57(12):1131-1135. The purpose of this paper is to present a detailed description of the effects of hypergravity on the mitogenic response of human lymphocytes to concanavalin A. The effect on cultures of lymphocytes isolated from peripheral blood are compared with those on whole-blood cultures obtained by diluting fresh blood with culture medium 1:10. Whole-blood cultures of lymphocytes from crew members will be investigated inflight on the Spacelab mission D-1 in 1985 and SLS-1 in 1987. In hypergravity there is an increase in lymphocyte activation of up to 500%. A similar increase can be induced by pre-incubating the cultures in hypergravity prior to exposure to concanavalin A at 1 G. The effect is less evident in cultures of isolated lymphocytes. The influence of autologous plasma and erythrocytes has also been investigated. Plasma and hypergravity have a synergistic and positive effect on lymphocyte activation, i.e. cultures of separated lymphocytes show the highest activation when incubated at 10 G and supplemented with autologous plasma. Conversely, erythrocytes depress lymphocyte activation.

Nachtman RG, Driscoll TB, Gibson LA, and Johnson PC Jr. Commercial over-the-needle catheters for intravenous injections and blood sampling in rats. *Lab Anim Sci* 1988;38(5):629-630.

Nachtman RG, Dunn CDR, Driscoll TB, and Leach CS. Methods for repetitive measurements of multiple hematological parameters in individual rats. *Lab Anim Sci* 1985;505-508. Methods have been developed which permit frequent repetitive blood sampling of rats without perturbing physiological parameters of interest. These techniques allow a comprehensive hematological study over several weeks, in individual rats, thus permitting full documentation of selected parameters during growth and development.

GRAVITATIONAL PHYSIOLOGY

Leonard JI, Leach CS, and Rambaut PC. Quantitation of tissue loss during prolonged space flight. *Am J Clin Nutr* 1983;38:667-679. An analysis of data from the three Skylab missions was performed to assess the lean body mass (LBM) and fat components of inflight body weight loss. Six methods for determining LBM were employed based on changes in total body water, total body potassium, nitrogen balance, potassium balance, and stereophotometric-body density. Those based solely on body potassium, and potassium and nitrogen balances (when expressed as shifts from preflight control), consistently overestimated LBM loss unless appropriate corrections were made. The average results from the various methods indicated that of a mean inflight total body weight loss of 2.7 ± 0.3 kg (SD) for all nine crewmembers, more than half (1.5 ± 0.3 kg) can be attributed to loss of LBM (including 1.1 kg body water), the remainder (1.2 ± 0.3 kg) being derived from fat stores. The reduction of LBM appeared to be complete after the first month of flight and thereafter was largely independent of mission duration, diet, and exercise.

Ross MD. The influence of gravity on structure and function of animals. *Adv Space Res* 1984;4(12):305-314. Gravity is the only environmental parameter that has remained constant during the period of evolution of living matter on Earth. Thus, it must have been a major force in shaping living things. The influence of gravitational loading on evolution of the vertebrate skeleton is well recognized, and scale effects have been studied. This paper, however, considers in addition four pivotal events in early evolution that would seem to have been significant for the later success and diversification of animal life. These are evolution of the cytoskeleton, cell motility (flagellae and cilia), gravity detecting devices (accelerometers), and biomineralization. All are functionally calcium dependent in eukaryotes and all occurred or were foreshadowed in prokaryotes. A major question is why calcium was selected as an ion of great importance to the structure and function of living matter; another is whether gravity played a role in its selection.

Spangenberg DB. Statolith formation in Cnidaria: effects of cadmium on *Aurelia* statoliths. *Scan Electron Microsc* 1986;4:1609-1618. Statolith formation in Cnidaria was reviewed with an emphasis on *Aurelia* statoliths. The review provides information on the chemical composition, mechanisms of initiation of mineralization, and effects of environmental factors on Cnidarian statolith formation. Environmental factors discussed include modified sea water ingredients, X-irradiation, clinostat rotation, and petroleum oil ingredients. A detailed account of the effects of cadmium on mineralization and demineralization of *Aurelia* statoliths is given. Cadmium at dosages of 2 to 4 μM significantly reduces statolith numbers in developing ephyrae. At a dosage of 3 μM , cadmium accelerates statolith loss in unfed ephyrae studied at 4 and 8 days following ephyra release from strobilae. Cadmium, therefore, is shown to reduce statolith numbers in developing ephyrae and to cause greater reduction of statolith numbers in unfed ephyrae after 4 and 8 days than occurred in controls. Supplementation of Cd^{2+} -containing artificial sea water (ASW) with calcium (3X and 5X ASW calcium content) results in higher numbers of statoliths at day 4 as compared with cadmium-treated ephyrae. At 8 days only the 5X calcium supplemented ASW is effective in enhancing statolith numbers in Cd^{2+} -treated ephyrae. These results suggest that cadmium competes in some manner with calcium at the mineralizing sites of *Aurelia*.

Spangenberg DB. Rhopalium development in *Aurelia aurita* ephyrae. *Hydrobiologia* 1991;IN PRINT. Rhopalium of developing ephyrae were examined using the SEM and TEM at 24 h intervals following strobilation induction. Kinocilia are shorter in the ephyrae stage than in polyps. A few ephyrae-type kinocilia are found in rhopalium as early as 24 h after induction, before a distinct rhopalium is seen. By 72 h, the shorter kinocilia predominate and are almost as numerous as in ephyrae (120 h). Many of the kinocilia are associated with mechanoreceptor cells (MR) found in the rhopalium. These MR cells are compared to those reported for medusae. Although newly-released ephyrae lack a touch plate, the MR cells in their rhopalium along with the statocyst and neuromuscular system apparently enable these organisms to detect and respond to gravity.



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